Intrapericardial Delivery of Omega-3 Polyunsaturated Fatty Acids

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Acknowledgements

Dedication

This dissertation is dedicated to Melissa Rolfes for her continuous love and support. I am grateful to have you as a wife and friend.

Thesis Abstract

Ischemic heart disease is a major cause of morbidity and mortality in the US, as well as other developed nations. An estimated 1,255,000 Americans will suffer from acute myocardial infarctions (MI) in 2012 alone [1]. Further, in 2008, this contributed to over 400,000 deaths from coronary heart disease [1]. Thus, many therapies have been investigated as treatments so to minimize damage after MI. Because of the unpredictable nature of acute MI, many of these therapies have been focused on reducing cell damage after the onset of symptoms, such as stenting, angioplasty and/or coronary artery bypass grafting. Associated arrhythmias can be common in such patients.

Atrial fibrillation (AF), is the most common arrhythmia, occurring in approximately 1% of the population [2]. While not immediately life-threatening, AF can have significant impact on the quality of life. This self-propagating disease progresses from paroxysmal (self-terminating episodes less than 7 days) to persistent (recurrent episodes lasting more than 7 days) to permanent AF. As with many cardiac diseases, AF sufferers generally respond better with early treatment.

Cardiothoracic surgery poses a great risk for generating associated AF. With elicitation rates of up to 25-40% in such patients, therapeutic or preventative treatments have gained much recent attention. Importantly, in addition to lengthening a given patient

time in the intensive care unit (ICU), postoperative AF may increase rates of postoperative stroke and thus the total cost of the patient care [3].

Intrapericardial delivery of anti-arrhythmic agents

The main goal of targeted drug development is to maximize effects on desired tissues while minimizing side-effects from drug accumulations in peripheral organs. In other words, localized therapies are designed to deliver and maintain high drug concentrations in or near the desired tissue. More specifically, such local deliveries often create or use natural barriers to contain the bulk of administered therapeutics in a specific region. These barriers minimize movements of the medication, keeping a large dose in the intended tissue with minimal crossover with the systemic circulation. The pericardial space surrounding the heart provides one such unique reservoir and potential therapeutic benefits have been investigated by employing several antiarrhythmic agents, including: esmolol [4], solatol [5], atenolol [5], ibutalide [6], procainamide [7], [8], digoxin [7], amiodarone [9], arachadonic acid [10], nitroglycerin [11] and L-arginine [12]. The intrapericardial (IP) delivery of each of these agents has been shown to have beneficial electrophysiological effects when delivered within the pericardial space in various animal models. Further, in those studies that measured plasma concentrations, they showed minimal crossover into the bloodstream [7–9]

Delivering therapeutics to the pericardial space has the potential to produce high tissue concentrations while minimizing crossover into the plasma. This local containment, in turn, reduces side-effects in other systemic organs, which can be a common problem with antiarrhythmic drugs. In other words, while current regimens using approved drugs

achieve their end desired affects with conventional oral or intravenous delivery, the needed high concentrations of these agents within the bloodstream transports them to all tissues. With IP delivery, however, not only are local concentrations increased, but tissue concentrations can more tightly controlled. This control results in fewer variables to affect tissue delivery, the therapeutic levels can be better titrated with feedback loops. This is especially beneficial with drugs that may have a narrow therapeutic window.

While numerous animal models and experiments have been performed with success, there has been limited translation to clinical use. This is likely due to a lack of clinical trials and experience. With new delivery techniques come new complications, and potentially altered effects. For instance with pericardial delivery, agents need to diffuse into the myocardium, rather than being perfused through the blood and reaching each cell. Thus drug pharmacodynamics must be understood with this new technique before clinical protocols can be established.

Thesis objective

The IP delivery of drugs is very promising, with positive results seen in numerous studies to date. Yet, before specific clinical protocols can be established, a drug and target must be identified, and methods developed and carefully verified. Typically, both preliminary work *in vitro* and *in situ* animal models must show positive results before clinical trials are initiated and subsequent human protocols can be put into practice. Parameters that need to be determined include drug dosing, delivery rates and pharmacodynamic studies. The primary purpose of this thesis is to evaluate the potential efficacies of

antiarrhythmic therapies delivered to the pericardial space so as to reduce susceptibility to arrhythmias during cardiothoracic procedures.

To accomplish these objectives, I began by specifically researching AF mechanisms and consequences of cardiac ischemia/reperfusion injury. *Chapters one* and *two* of my thesis incorporates this initial experimentations and learning with atrial fibrillation as well as localized drug therapies. These chapters summarize my initial background work that serves as the foundation for additional investigations. In *chapter three* of my thesis, I delve further into localized therapeutic delivery and make use of it in a porcine model of perioperative AF, treating with metoprolol directly in the pericardial space. The remaining chapters focus on the pericardial delivery of omega-3 polyunsaturated fatty acids. These chapters consist of the studies that make use of knowledge and experience gained in the first few chapters, then focuses on the effects of omega-3 polyunsaturated fatty acids in several potential clinical applications. These chapters make use of a surgical swine model, as well as exploring fatty acid delivery for preconditioning a donor heart prior to transplant transportation and post implant. In the final section of my thesis I will summarize the potential benefits of the pericardial delivery of therapeutic agents and discuss the future investigations needed to make these approaches fully translations into a clinical setting

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Preface

As I started my research into cardiac disease and atrial fibrillation (AF), the human heart library within the visible heart lab became a valuable resource. I began an investigation into anatomical changes during AF, measuring volumes and wall thicknesses in diseased and healthy hearts. These measurements served to further my knowledge and understanding of AF. This, along with the accompanying research into the disease progression and treatment, served to form much of my knowledge and understanding of cardiac anatomy and physiology.

Summary

Background Atrial fibrillation (AF) causes a continuum of atrial anatomical remodeling.

Methods Using a library of perfusion fixed human hearts, specimens with atrial fibrillation were compared to controls. During this preliminary assessment study, direct measurements were taken of atrial volume, pulmonary vein (PV) circumference, and left atrial wall thicknesses.

Results Hearts with AF typically had larger atrial volumes, as well as a much larger variation in volume compared to controls (range of 59.6-227.1mL in the AF hearts compared to 65.1-115.9mL in control). For all hearts, right PVs were larger than left (mean 171.4 ± 84.6 for right and 118.2 ± 50.1 for left, $p < 0.005$). Left atrial wall thicknesses ranged 0.7-3.1mm thick for both AF and control hearts.

Conclusions Hearts with AF had a large range of sizes which is consistent with the progression of atrial remodeling during AF. The large range of thicknesses will influence the amount of energy needed to create transmural lesions during ablation procedures.

Keywords: Left atrial dimensions, volumes, pulmonary vein ostia, atrial fibrillation

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Introduction

Atrial fibrillation (AF) is the most prevalent tachyarrhythmia, with a prevalence of 1% in the general population [1]. One of the particular clinical features of this arrhythmia is its self-perpetuating nature. Paroxysmal AF (self-terminating episodes) may eventually turns into persistent (> 7 days) and then even permanent AF [2-4]. This progression is considered in part, due to both structural and electrophysiological remodeling of the tissue that provide substrates for maintenance of such arrhythmias. Structural remodeling associated with AF refers to physical changes, such as dilation of the atria and interstitial fibrosis. The electrophysiologic remodeling results the shortening of the atrial effective refractory periods, thus aiding the perpetuation of an arrhythmia and/or limiting the ability to terminate fibrillations. More specifically, the self-perpetuation is especially disturbing symptom when patient long-term prognosis is taken into account: mortality rate doubles and stroke occurrence averages up to 5% per year in patients with AF, which is 2 to 7 times the rate of individuals of similar ages whom are uninflected with AF [4].

The prevalence of atrial fibrillation in relation to age and associated risks

The clinical treatment of AF is an especially relevant topic in geriatric cardiology due to the well documented increasing prevalence with age. When considering an overall general population, the relative prevalence of AF is 1%, yet in persons over 40 years of age the prevalence reaches 2.3% and then climbs to 5.9% for individuals over 65 [5]. The geriatric population, defined by the WHO as persons with age greater than 65, contains over 75% of people suffering from AF [1]. It should also be noted that AF has also been found to be more common in men than women (1.1% versus .8%) [1].

Atrial fibrillation is well known to be associated with decreases in quality of life: as reported by as many as 68% of patients with paroxysmal AF [6]. Interestingly the psychological, not physical, quality of life may be impacted more [7]. In addition to stroke [4], AF has also been associated with many other health problems that would cause decreases in quality of life such as depression, professional and sex life complications [8], etc. However, it is debated whether or not AF is or is not associated with cognitive decline [9-11]. It is highly probable that because AF causes an increased risk of stroke and emboli, that cognitive decline in part be linked to the potential for multiple small and transient cerebral infarcts, yet this will also be dependent on the given patient and their anticoagulation management [12].

In addition to the above detrimental effects of AF, anatomical remodeling of the atria can occur within the heart. Patients who have AF tend to have larger left atria and larger pulmonary veins which could potentially lead to the further propagation of AF [13]. Alternatively, the reverse has also been shown: upon return to sinus rhythm after radiofrequency ablation, there is a measureable reduction in left atrial size [14,15]. Depending on the length of AF and the stage of remodeling, patients with the arrhythmia may have large variation in their anatomy.

Left Atrial volume measurements

Heart left atrial (LA) volumes have been selected here to be measured in this sample of perfusion fixed human hearts, due to reported AF related atrial remodeling (dilations). For example, the work by Leung et al. found that increases in LA volumes could be used

to independently predict the increased risks of cardiovascular death, heart failure, AF, stroke, or MI [16]. Further, subsequent reductions in LA volumes (reverse remodeling) has been found to be a strong predictor of the successful treatment of AF using either catheter based [17] or surgical [18] ablations. Relative LA volumes for a given patient have also been found to predict the potential for recurrence of AF after cardioversion [19] and conversion of atrial flutter to AF after successful ablation [20]. More specifically, the probability of relapse after catheter ablation was found to be significantly higher for LA volumes greater than 145 ml [17]. LA volume index greater than 135 ml/m^2 was found to have 100% specificity and LA diameter greater than 60mm was found to have 100% sensitivity for prediction of surgical Maze failure [18]. Not surprisingly, normal LA volumes have been found to be associated with absence of thrombus [21] and relative LAA dimensions have been found to be a positive predictor for stroke/TIAs in patients with AF [22].

Pulmonary vein sizes

Since the clinical discovery that the pulmonary veins (PVs) can be substantial site-sources of ectopic beats [23], PV anatomy has attracted increasing attention. A patient's pulmonary vein sizes are useful measurements when planning for an ablation since some procedures, such as cryoballoon ablation, offer multiple sized devices to optimize PV isolation. The PV circumference was chosen because even though the PVs (especially the left PVs) are typically oval in shape [24], they are also considered as compliant and a balloon pushed against the ostium will slightly change its shape. In other words, using simply the long or short axis for planning may lead to the decision to use a balloon that is too big or small, respectively.

Left Atrial Wall Thicknesses

Left atrial wall thicknesses are also commonly assessed and were done so here as well. The area on the posterior wall near each of the PVs was measured as well as the center of the posterior wall. These locations were selected not only because they are smooth in comparison to the left atrial appendage, but they are often areas of therapeutic focus during AF ablation procedures. Variation in LA wall thicknesses is also important to consider during radiofrequency ablation procedures, since it should influence the amount of energy to be applied [25].

One of the primary goals of this preliminary assessment study is to present our ongoing investigations as to human cardiac anatomy. Our Visible Heart[®] Laboratory has a current library of over 200 human heart perfusion fixed specimens that we can uniquely employ for such investigations. In addition, many of our obtained images are and videos of functional cardiac anatomies are available to the general public, via our free-access web site, "The Atlas of Human Cardiac Anatomy" (http://www.vhlab.umn.edu/atlas).

Methods

Human hearts deemed not viable for transplantation were donated for educational and research purposes. Following similar guidelines for transplantation, the hearts were stopped, cooled and transported to our lab. Within 24 hours of being excised, these specimens were weighed and the aorta, superior vena cava, pulmonary artery, pulmonary veins and the inferior vena cava (when possible on a given specimen) were cannulated and attached to a perfusion fixation chamber as described previously [26]. This approach will preserve the hearts in a modified end-diastolic state (fully expanded atria and ventricles). These hearts were fixed with 10% formalin in PBS solution for at least 24 hours under normal physiologic pressure and then stored in formalin.

Heart specimens have been collected and added to our library since 1997 and to date the library has grown to over 200 in the collection. Anatomical studies can be performed on this large collection so to better understand the variation in anatomy between different hearts. For most of such specimens, pertinent clinical histories are also available, thus allowing us to assess the variation in anatomy with respect to the relative disease state of the heart. In the present study, we identified and used hearts that had a clinical diagnosis of AF and compared them to hearts with no indication of AF or mitral/tricuspid regurgitation (normal anatomies). The AF hearts were sex, age, weight, and height matched to control hearts. For all but 2 of this sample of 10 AF hearts, acceptable control hearts were found and/or were measureable (Table 1.1).

	AF	Control
n	10	8
age (sd)	69.9 ± 11.6	63.9 ± 12.5
male (%)	6 (60)	4 (50)
weight (kg)	80.8 ± 21.8	93.5 ± 31.5
CAD (%)	2 (20)	0 (0)
HTN (%)	4 (40)	5 (62.5)
Valve insufficiencies	0	0

Table 1.1: Patient information from atrial fibrillation and their corresponding control hearts. For all but two AF hearts suitable matches were found as controls based on subject genders, ages, weights and heights for these collected specimens.

To measure the volumes of the atria, the hearts were oriented such that the annulus of the atrioventricular valves would be parallel to the ground while measuring their respective side. To measure the volume of the left atrium (LA), the aorta and all but one PV were clamped off and the LA and left ventricle were filled with deionized water. After the LA was completely filled the water was drawn out and collected until the water level reached the level of the annulus of the mitral valve, which was determined by visualization using a fiberscope. The weight of the water was taken and this process was repeated a minimum of three times. The right atrium (RA) was measured the same way; however the pulmonary artery, inferior vena cava, coronary sinus and posterior interventricular vein were closed up and the water was drawn out to the level of the tricuspid valve. The siphoned water was weighed and repeated three times. Note, that since deionized water was used, the weight of the fluid is equivalent to the volume of the chamber.

All of the other anatomical measurements that were recorded were obtained using standard calipers or a C-clamp micrometer. The thicknesses of the LA were determined using the C-clamp at five distinct locations: the center of the posterior LA wall and the junction of the right superior, right inferior, left inferior and left superior PVs. The junction of a PV was defined as the location in which the vein transitioned into the wall of the left atrium. To determine the relative areas of the PVs, the opening of each vessel was pinched together to measure half of the circumference (Figure 1.1) and obtain an area assuming the vessel is circular. Although it has been found in the literature that the PVs are generally oval in shape, it was decided that comparing the vessels as circular would alleviate the problem of re-approximating the oval shape of the pulmonary veins. Furthermore, in some of these cases, the PV can be slightly distorted due to the nature of the fixation process.
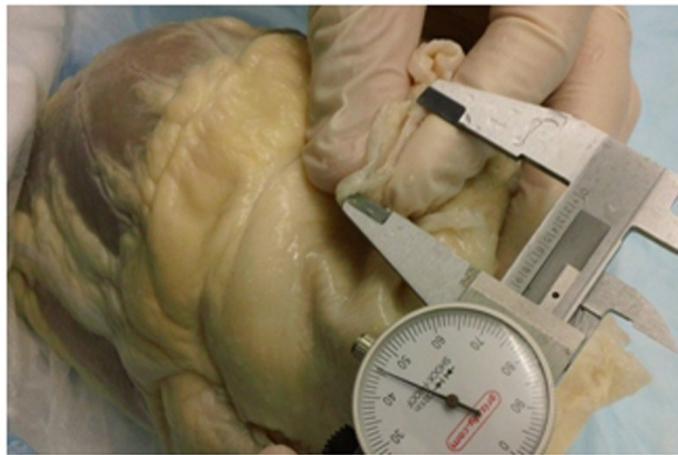
Figure 1.1: Illustration of the half circumference measurements taken to obtain diameters and area measurements of the various PVs.

The size of the fossa ovalis (FO) in each specimen was also measured; it should be noted that since the FOs were not collapsed during the fixation process, we were able to

obtain accurate assessment of these structures. These areas were determined by measuring the diameters along the superior/inferior and the anterior/posterior direction of each FO.

Values presented are mean with standard error unless otherwise noted. For comparisons of continuous variables between two groups a Student's t test was used.

Results

Within these investigated hearts, as expected the AF hearts had larger LA, RA and total atrial volumes compared to the controls hearts (Figure 1.2). However when we compared individual AF heart to their specific sex, age, weight and height matched control, there was no clear correlation as to which types of heart had larger atrial chamber sizes. Yet, the data did suggest that there was a higher variability within the hearts from the AF patients compared to the controls: with a range of 59.6ml to 227.1ml in the AF hearts compared to 65.1ml to 115.9 ml in the control hearts. Note that the differences in ranges for both atria are over 3 times larger in the AF group than in the control group.

	All PVs		Right PVs		Left PVs	
	Mean (mm^2)	Median (mm^2)	Mean (mm^2)	Median (mm^2)	Mean (mm^2)	Median (mm^2)
AF	152	139	193	170	116	109
Control	138	120	156	126	121	100

Table 1.2: The mean and median values of the pulmonary vein ostia between AF and control heart specimens.
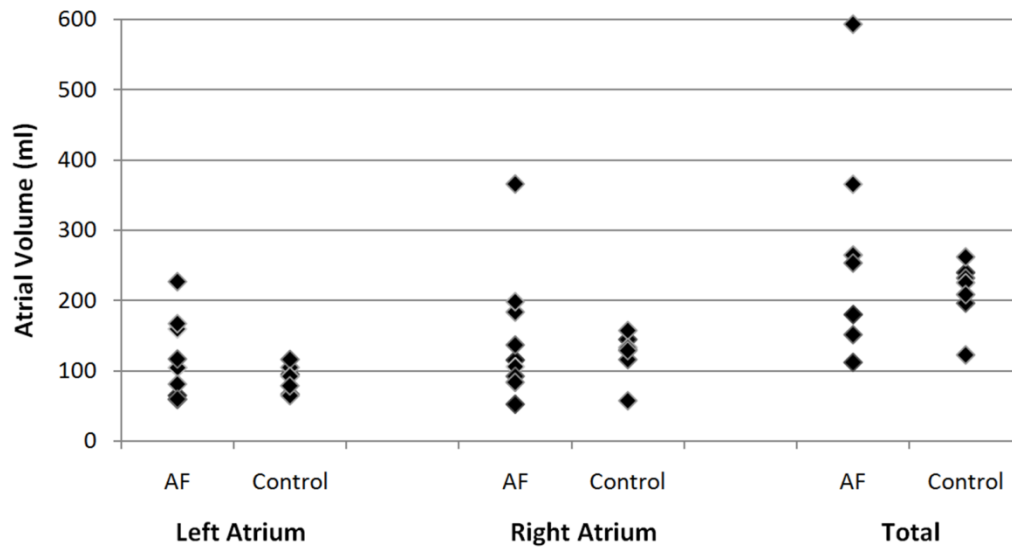
Figure 1.2: Comparisons of the paired left, right and total atrial volumes between the matched AF and control hearts. Note that the AF hearts had greater variability of volumes compared to the controls.

As noted above, the sizes of the pulmonary veins were assessed by assuming all as circular openings (Figure 1.3). Between the two groups, the control hearts had smaller PV ostia for both the left and right veins. One can observe two different peaks on these histograms; with the higher peak associated with the data from the AF hearts. This finding was consistent also when the right and left PVs were compared separately (Figure 1.3). Interestingly, there was also the observed trend that the left PVs were smaller than the right PVs in both the control and AF specimens (Table 1.2). Further, when trying to determine that the right PVs were larger than the left, while looking at the whole population of hearts, there was a significant difference seen between the two (mean 171.4 ± 84.6 for right and 118.2 ± 50.1 for left, $p < 0.005$).

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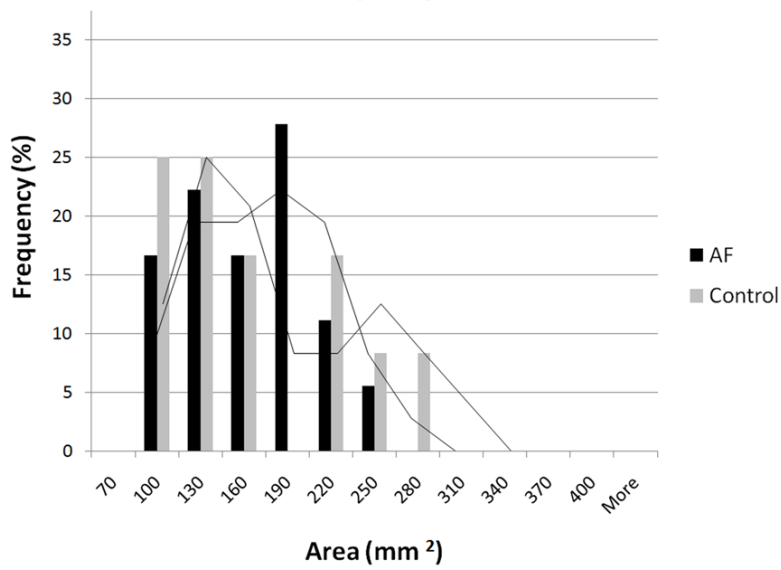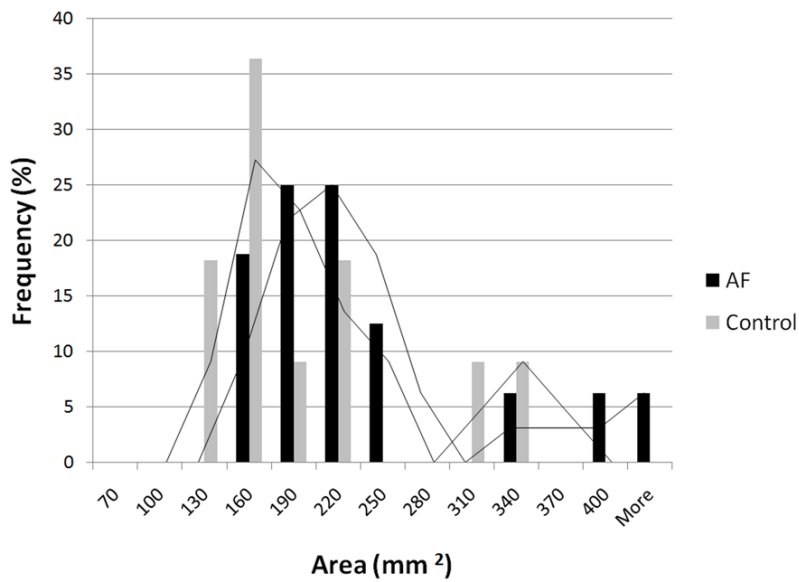
Figure 1.3: The relative variation in pulmonary vein ostia sizes between the two groups of heart specimens. The histograms provide the corresponding areas of these ostia. Top: the relative distributions of pulmonary vein sizes of the right pulmonary veins; with means of 24.2mm2 and 21.6 mm2 and medians of 23.1 mm2 and 19.9 mm2 respectively. Bottom: the left pulmonary vein areas, means of 18.7 mm2 and 19.0 mm2 and medians of 18.4 mm2 and 17.7 mm2 respectively. The trend lines depict the two point moving averages of the distributions and illustrate the relative differences between these two heart populations.

Although there were observed trends between the relative areas of the PV ostia of these

two groups, in contrast there were minimal differences in the thicknesses of the LA walls

within our samples: the means and medians were 1.19mm and 1.24mm for the AF

population and 1.26mm and 1.21mm for the control population, respectively. The

histograms and population spreads between the AF and control groups appear to elicit a

fair amount of overlap; in other words, they appeared similar between the groups and

were not dependent upon the diagnoses of AF (Figure 1.4). Similar findings were also

observed when examining the relative sizes of the FO in these hearts. The FO

dimensions only had slightly larger sizes in the anterior-posterior (AP) vectors, whereas

they elicited minimal or no differences in the superior-inferior vectors (SI) (Figure 1.5).
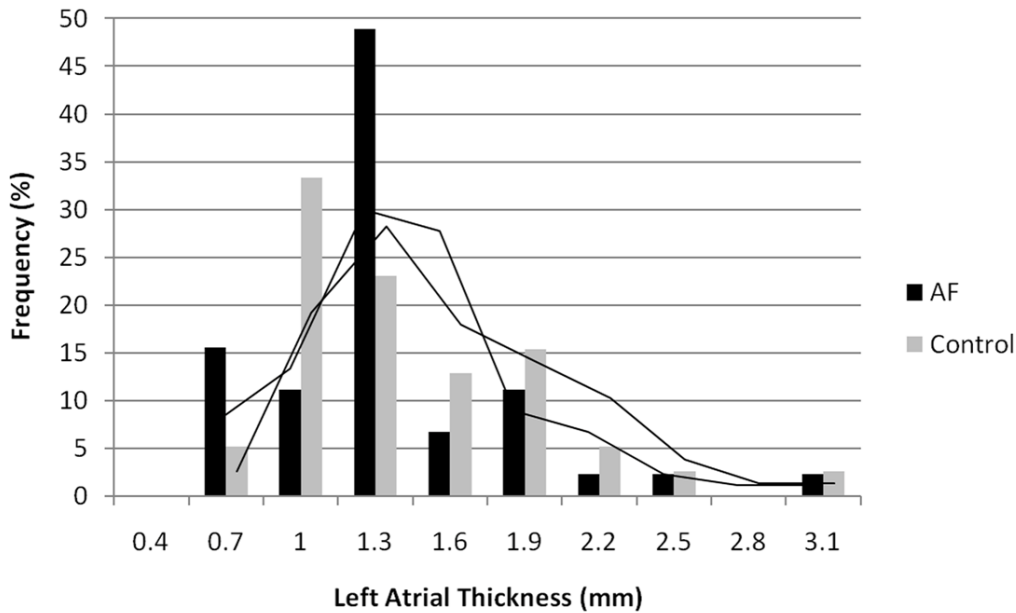
Figure 1.4: Relative distribution of thicknesses of the left atrium between the AF and control hearts. These thicknesses values were determined by taking measurements from each of the four pulmonary vein junctions in each heart, as well as the center of the posterior walls of the left atria. The trend lines depict the two point moving averages of the population distributions.
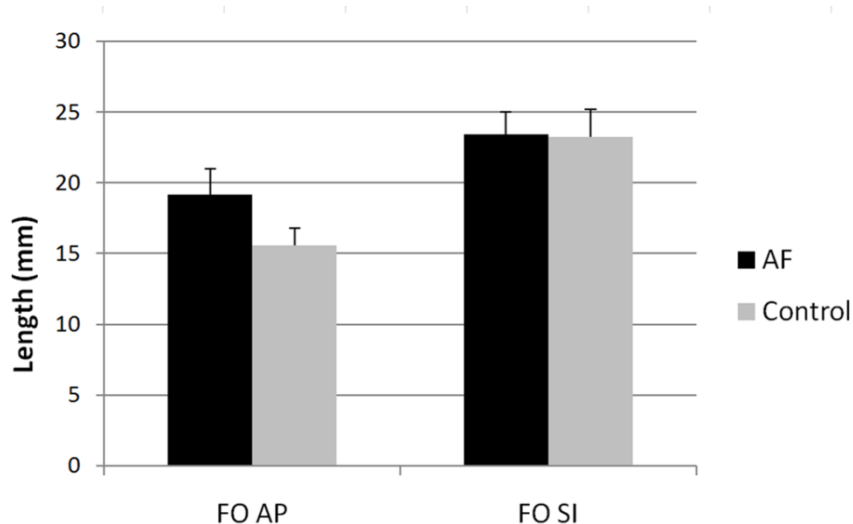
Figure 1.5: Average widths and heights of the fossa ovalis sizes between the two populations of hearts. The relative widths of the fossa ovalis were designated as the fossa ovalis anterior-posterior (FOAP) measurements and the heights were designated as the fossa ovalis superior-inferior (FOSI) measurements (error bars represent the SEs). It was observed that there were no significant differences between the calculated averages of the measurements taken of and the distributions of the sizes showed similar trends between the AF and control hearts (data not shown).

Discussion

In the present study we describe novel measurements of relevant cardiac anatomies obtained from our unique library of perfusion fixed human hearts. In this preliminary assessment study, we describe approaches to allow for the detailed quantifications of specific atrial anatomies. The presented information provides useful insight as to the changes in structure that may occur when the human heart remodels following the development of AF, and we plan to continue these studies as our relevant library of specimens grows.

Current imaging modalities, including MRI, ultrasound, and CT scans, allow for relative approximations of the volumes and sizes of the LA and RA using algorithms available in various software packages or by simply estimating diameters. Yet, it is considered that

measurements of LA volumes are more accurate than diameter data, especially as a given heart dilates, i.e., small increases in diameter will provide for larger increases in derived LA volumes [27,28]. Our volume measurements take into account the variability of chamber anatomies from heart to heart, and thus resulted in a more accurate representation of the total chamber sizes. Therefore, these chamber volumes, along with thickness measurements of the LA, were considered to better point to variations in anatomies due to a given pathological state. However based on our data on this somewhat small sample size, we could not make correlations as to the clinical state based solely on these measurements. Yet, it should be noted that even in the absence of AF, large atria are often indicative of other cardiovascular issues [29], thus in these hearts we studied from this elderly population of subjects it was likely that the non-AF hearts, or so-called controls, may have been not truly normal.

Our initial characterization of these AF hearts confirmed various trends that have been reported within current literature, such as the increased sizes of right PVs compared to the left PVs [13]. However, as with most anatomical studies a large sample set is required so to potentially produce statistically significant results. With our growing library of these perfusion-fixed human hearts, we will be able to perform ongoing anatomical studies so to better assess the differences within various populations of heart disease states or demographics. Our collection of specimens to date has allowed us to obtain useful insights as to the common anatomical features of the atria from patients with AF with a high degree of accuracy. The variety of physical specimens also allows for physician education and therapeutic device feedback as any number of devices can be placed within the hearts. The continued study of the details of human cardiac anatomy

will provide further insights as to the changes in structure that may occur when the human heart remodels or reverse remodels due the presence of disease or following treatment, respectively. Though above study shows that PV measurements are not sufficient to diagnose AF, it illuminates the variety of anatomies that accompany the arrhythmia within the population. This large variation observed in the diseased patients is important for the physicians who treat AF, the educators, and engineers who must design medical devices to fit the whole range of anatomies. Using the fixed hearts we plan to continue to employ the study methods described here to further build an anatomical database, which we hope to compare to MRI, CT and 3-D models derived from each.

Conclusion

Atrial fibrillation comes with a great deal of anatomical and physiological changes. Anatomically, the size of the atria can vary greatly, but the pulmonary veins and vena cavae do not remodel significantly. The variation in anatomy has great importance and should be considered when treating and designing devices for AF.

Chapter 2: Localized Drug Delivery for Cardiothoracic Surgery

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Preface

With a solid foundation in cardiac anatomy and physiology, I continued my research into

AF. While investigating novel aspects of AF and its treatment, intrapericardial (IP)

delivery of antiarrhythmic drugs provided opportunity for innovation and continued

research. Local delivery to the pericardial space also has the potential to reduce the

variability seen with more conventional delivery routes, as seen in the GLP-1

pharmacokinetics (study included in appendix).

Chapter two of my thesis begins to focus on localized delivery of drugs*,* providing an

overview of targeted drug delivery, with a specific focus on thoracic surgery. It reviews

several areas where targeted and localized drug deliveries have the potential for major

improvements in organ and patient health. A particular focus is given on drug delivery to

the pericardial space. This chapter has been submitted for publication in the book

Thoracic Surgery, ISBN 980-953-307-096-9, to be published in the fall of 2012

Summary

Localized and targeted drug delivery methods have become expansive areas of research in a number of fields including cardiac research. These methods have the potential to increase therapy levels at targeted tissues while minimizing systemic exposure, and thus reducing side-effects. The focus of this chapter is to consider the local delivery of therapeutic agents to the patient's heart during a given surgical procedure. Yet prior to the discussion of such, one needs to consider typical routes of delivery and the validation studies required to confirm their utilities. This chapter outlines opportunities for delivery as well as therapies that are currently being developed to treat them. Within the research field, cardiothoracic surgeons are uniquely positioned to take advantage of these new technologies as they emerge thus increasing the effectiveness and safety of the therapy while improving post-operative outcomes.

Keywords: targeted delivery, local delivery, pericardium, cardioprotection

Introduction

It is noteworthy to consider that extensive bioavailability and bioequivalence studies are typically required before new drug therapies can be approved [1]. These studies include pharmacokinetic studies that take into account: 1) dosing, absorption and elimination rates of the drug and its active metabolites, as well as 2) the potential effects of multiple doses, drug interactions, and the differences whether medications are taken with or without food. A major therapeutic factor that compounds the variations often seen from patient to patient is individual differences in absorption and elimination rates. This will also cause variations in the amount of drug that reaches the desired targeted tissue when used as a clinical therapy.

While oral administrations are common and the easiest means to deliver outside of a hospital or clinical setting, intravenous (IV) delivery can eliminate some of the aforementioned patient to patient variability by bypassing the ingestion and absorption into a patient's bloodstream. However, a major obstacle with either of these delivery methods is that once a drug is in the blood plasma, the medication will circulate throughout the patient's body, not only reaching the intended site, but unintended sites as well. Hence, this will greatly increase the possibilities of causing unwanted side-effects. Thus, it is required that side-effects on each and every tissue be well described when therapeutic levels of the medication are administered.

Importantly, many drugs have described narrow therapeutic ranges. Slight increases in levels could cause severe undesired effects, whereas slight decreases often eliminate any therapeutic benefit. We describe in detail here how the targeted and local delivery of

medications may overcome many of these obstacles in traditional delivery methods by simplifying the pharmacokinetics, reducing variability and allowing higher doses to reach the intended target.

Targeted Drug Delivery

"Targeted drug delivery" is a general term that describes a variety of methods that can be used to increase the concentrations of a given drug at a primary location within the body relative to others body tissues. Often called "smart therapies," targeted delivery includes methods such as antibody labeling, ultrasonic release and/or localized delivery that can increase drug concentrations at the desired tissue. The primary intent is to increase the intended beneficial effects while reducing side-effects.

Developments in targeted drug delivery were commonly pioneered with anti-cancer drugs. These treatments are often highly toxic and have undesirable side-effects, which in turn can greatly reduce quality of life and limit the dose levels that can be administered. Therefore, if the levels of these drugs can be increased specifically at the site of a tumor relative to the rest of the body, the same or reduced dose levels will have much greater effects at the site of the cancerous tumor.

One commonly described method for accomplishing such is creating or adding components to cancer drugs that preferentially bind within the tumors. More specifically, the identification of differences in endothelial surfaces in the growing tumors has led to the development of cancer medications that preferentially adhere to the endothelial surfaces within the vasculature of the tumor thus, increasing the desired effects. This

results in lower exposures of non-target systemic tissues to the drug than were previously possible. In a similar manner, numerous biomarkers have been identified that become upregulated in diseased cardiac tissue, and therefore have become targets in emerging therapies [2], [3]. Alternatively, drug treatments can be encapsulated in such a way that they are released at the desired location, such as where triggered by high frequency ultrasound, causing focal increases in drug concentrations [4–6]. Table 2.1 summarizes many currently used and investigated targeted therapies, several of which are described in greater detail throughout this chapter.

Delivery Method	Advantages	Disadvantages
Targeted Drug Delivery		
Ultrasound or heat disrupted carriers	non-invasive focal treatment to potential asymmetric areas	equipment intensive, potential buildup within liver and spleen
Biomarker targeted	simple administration	designer molecules need to be created, approved
Localized Drug Delivery		
Pericardial delivery	entire epicardium treated, well contained, easy access in surgery	invasive, pericardium often left open after surgery
Direct myocardial/tissue injection	increases myocardial concentrations, long lasting	invasive/minimally invasive
Drug eluting wafers	long or short lasting, tunable degradation	minimal migration small doses, reliant on resorbable wafer or must be explanted
Implantable pump	local drug delivery on demand, larger continuous dosing possible	invasive, needs refilling, shortcomings associated with implantable devices
Coronary injection	increases myocardial concentrations	invasive/minimally invasive, treatment still enters blood

Table 2.1: Various targeted therapies and their advantages and disadvantages.

Localized Delivery

"Localized drug delivery" is defined as a specific form of targeted delivery where the

medication is given at a certain site which allows for reduced movement and subsequent

absorption into the bloodstream. Localized delivery is often provided to a naturally

enclosed space, such as the bladder or into the vitreous humor of the eye, but other

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techniques can limit movement such as a gel or a patch. In general, by delivering a

given pharmacological treatment to a specific target tissue site via an implantable pump

or acute access, localized therapy will have reduced systemic effects on peripheral

tissue thereby limiting side-effects, while maintaining increased control.

Just as IV delivery increases control and decreases variability over oral delivery, by

eliminating the gastrointestinal tract, local delivery increases control and decreases

variability by eliminating reliance on one's circulation for distribution. Thus localized drug

delivery carries the potential for both increased effectiveness of treatments, while

reducing the quantities needed (Figure 2.1). These reductions have important applied

implications when one employs either drug pumps or impregnated gels to delivery

therapies, as they can only hold limited volumes.
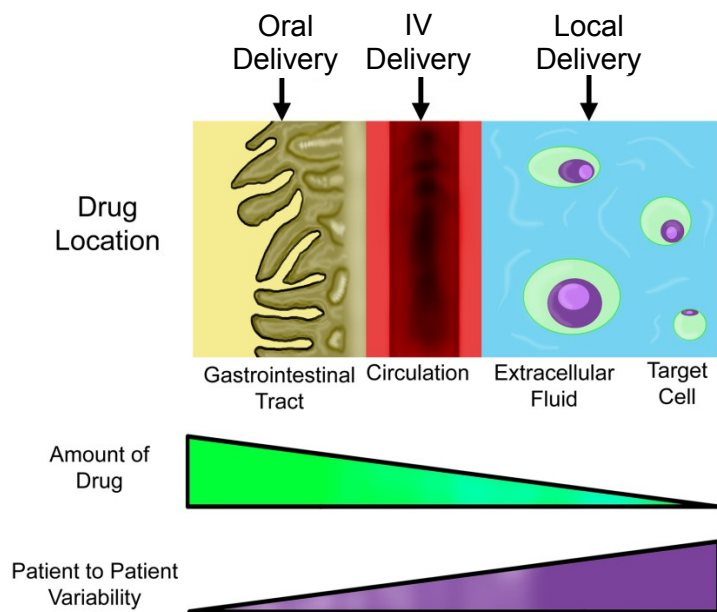
Figure 2.1: Schematic of differences in drug delivery by oral, IV or local delivery modalities. Increasing the number of transport steps increases the amount of drug necessary and patient to patient variability increases at each step.

Finally, an additional benefit often seen with localized delivery is a relative increase in the therapeutic drug half-lives. For example, since localized treatments typically have minimal crossover with the patients' circulation, there is limited exposure to their livers and kidneys, which are typical sites for drug metabolism. Thus the relative therapeutic half-lives of many pharmaceuticals will be increased, which is another mechanism that could also decrease the amount of a given agent required to achieve sustained therapeutic dose levels [7].

While targeted drug delivery is the focus of broad research, it is our intent with this chapter to narrow the focus more specifically on localized therapy and the unique

opportunities provided by the access obtained during thoracic surgery. For instance the pericardium surrounds the heart and provides a unique enclosed volume, in which one can target the myocardial epicardial surfaces. In other words, the localized drug delivery to the pericardial space will allow the agent to diffuse into the myocardium while reducing those amounts present in the circulating blood. During cardiothoracic surgery one has unique access to this otherwise difficult to reach space where subsequent therapy can be delivered throughout the perioperative period. Therefore, as therapies emerge to treat heart failure, local delivery during thoracic surgery is positioned to be a viable therapeutic option that can be delivered with minimal added time, few complications or complexities. At the same time it has the potential to reduce ischemic damage and arrhythmias, and hold great potential for improved outcomes, reduced morbidities and increased cardiac health.

Known procedural complications and potential therapeutic opportunities:

Cardiac surgery is typically defined by a broad class of surgeries intended to correct heart problems. They can be broken down into several categories that include: 1) valve repair and replacements, 2) structural heart repairs, 3) implantations of devices for either the maintenance of rhythm or mechanical performance, and 4) heart transplant. Today, the most common cardiac surgery is coronary artery bypass grafting (CABG) [8]. Despite the recent trend towards minimally invasive cardiac procedures, there still remains a significant need for open surgical techniques. Due to the invasive, and many times emergent, nature of these procedures they are also excellent candidates for targeted

delivery (it should also be noted that many minimally invasive and scheduled procedures could also take advantage of target therapeutic delivery of various agents).

It is generally accepted that open cardiac surgery is not without potential complications. On the other hand, the occurrences of such complications can be viewed as unique opportunities for introduction of localized drug delivery to improve patient outcomes. It is important to note that complication rates and outcomes can only be truly assessed on both procedural and patient population bases; yet there are often common complications across all classes of cardiac surgery. Statistics on the following procedural complications and proposed treatments using targeted delivery will be discussed: neurologic, arrhythmic, gastrointestinal, bleeding, and infection. These complications when present will impact both short- and long-term morbidities and mortalities, i.e., influence lengths of hospital stays, quality of life, as well as financial burdens on both patients and healthcare providers.

To begin with, neurologic complications such as stroke, postoperative cognitive deficit, encephalopathy, and transient ischemic attack are of particular concern because of their much feared, potential long-lasting effects. Due to the nature of cardiac surgery and cardiopulmonary bypass, it can be foreseen that thrombosis or air emboli may occur from time to time, potentially causing these aforementioned complications. Poor neurological outcomes may also be caused by or related to arrhythmias, bleeding, or other complications. For example, prospective studies on the outcome of CABG patients show that the expected stroke rates vary from 1.5% [9] to 5.2% [8]. A broad range of disorders fall under the umbrella of post-surgical encephalopathy, a syndrome stemming

from many causes. Its prevalence has been described to vary based on the definition, with as many as one third of patients eliciting an encephalopathy post-operatively [10], but prospective studies show that by day four the number is reduced to 11.6% [11] indicating a transient nature. Expectedly, these complications occur more frequently in combined procedures and/or more technically challenging cases.

It should be mentioned that numerous modern surgical techniques, such as the no-touch aorta technique, have attempted to mitigate such neurologic complications. However, it will likely remain that current advances in surgical techniques may only reduce, but not totally eliminate these undesired events. Nevertheless, one can envision the added use of tailored drug cocktails with various procedures that could directly target the heart (pericardial space) or mixed within the blood returning from cardiopulmonary bypass. To date, potential therapeutic agents which have been shown to be effective have included:1) oxide scavengers 2) nitric oxide inhibitors [12], 3) agents to inhibit glutamate related neural excitotoxicity [13], and 4) the administration of aprotinin, a serine protease inhibitor [14]. It is possible that prior to patient rewarming, agents similar to tissue plasminogen activator could be administered in a manner that targets or localizes only the cerebral circulation and potentially destroys clots that were formed, thus mitigating negative effects prior to rewarming.

It is generally considered that atrial fibrillation is the most common complication to occur after cardiac surgery, occurring in up to 30% of cases [15]. A recent meta-analysis of 94 trials by Burgess and colleagues has shown that the five most common interventions were all effective at reducing incidence of atrial fibrillation, but to varying degrees. These

treatments included beta-blockers, sotalol, amiodarone, magnesium, and atrial pacing. It should be noted that amiodarone was the only intervention that was reported to reduce stroke on its own. Nevertheless due to the well documented negative systemic effects of such antiarrhythmics, several studies have begun to investigate the use of localized delivery to the pericardial space, discussed in more detail below. On the other hand, it should be noted that the systemic administration of modified agents could also prove both effective and avoid side-effects if targeting functionality was incorporated into the drug molecule. For example, this could be done by adding cardiac specific antibodies or ligands to therapeutic molecules. Nevertheless, such newly developed drug isoforms will require additional regulatory approval, perhaps only after clinical studies prove them safe and effective.

Gastrointestinal complications after cardiac surgery are less common, but importantly are associated with high rates of morbidity and mortality. Reported incidences are in range of <1% but mortality associated with this complication were approximately 25% versus approximately 3% without gastrointestinal complications [16], [17]. The most common of these complications include: upper intestinal bleeding, intestinal ischemia, acute pancreatitis, and perforations.

Despite the use of modern-day antibiotics, infection still remains a prevalent problem in cardiac surgery, as does administration of blood products. The two are commonly interrelated and it has been shown that transfusions of packed red blood cells(PRBCs) are significantly associated with postoperative infections [18]. However, this correlation does not necessarily imply causation, and the use of PRBCs may be related to

procedural difficulties, which may be the real underlying causation of such infection rates. It should also be considered that stored red blood cells also do not function normally and therefore, PRBCs may contain inflammatory and immuno-mediating substances. It was reported in a CABG patient series study at Duke University, that as many as 39.5% of patients received PRBCs [18]. Furthermore, out of the entire series, the postoperative infection rate was found to be 6.2% and specifically for those patients whom received PRBCs, they elicited infections rates ranging from 6-8.1% versus 5.1% for those who received no PRBCs [18]. Additionally, in a recent five year study on long-term survival following transfusion for CABG procedures, it was observed that patients not transfused have an approximately 2.5-fold better survival rates than those that did [19].

Interestingly, it has been proposed that actively targeted treatments could possibly restore normality to the RBCs prior to administration; such treatments could also incorporate anti-infection components. As mentioned above, RBCs can change drastically and immediately upon storage. It is considered that due to the low oxygen environment within the blood collection bag, RBCs switch to anaerobic metabolism, which in turn leads to lactic acid buildup and an overall reduction in blood pH. Furthermore, it is known the RBCs lose their signature bi-concave shape and become more spherical with storage: recall that it is this bi-concavity that is necessary for the cells to efficiently travel through capillary networks. Stored RBCs will also form aggregates due to activated surface proteins by crosslinking fibrinogen between GPIIb/IIIa binding sites [20]. This crosslinking increases the longer PRBCs are stored and potentially could contribute to neurologic complications and thus post-operative

cognitive deficits. Therefore, one could consider that prior to infusion of PRBCs, a targeted drug cocktail could be added to prevent or break these fibrinogen crosslinks and/or suppresses immuno- and inflammatory effects.

Finally, one should also consider that infection rates could potentially be reduced by localized drug delivery at the end of a given cardiothoracic surgical procedure. For example, one such method might take form of an antibiotic that could be sprayed on or within the thoracic cavity immediately prior to and after closure of the surgical entrance site. Such an application method may also have the potential to reach interstitial areas that are at risk for infections, specifically those with inherently minimal blood flows; in other words, these tissue areas would otherwise receive minimal amounts of orally or intravenously administered antibiotics.

Other drug targets

With recent advances in microfabrication it is feasible to locally deliver drugs in ways that were previously not possible. The field capsule endoscopy is a good example of these technologies and also one that may be further miniaturized and exploited for drug delivery. "Capsule endoscopy" is the swallowing of a pill with a video camera inside. The pill travels through the gastrointestinal system and records its journey. Even more sophisticated versions of these devices are being developed to be actively propelled by magnetic energy [21] or flagella type [22] propellers. It should also be noted that magnetic pills for drug delivery are currently under development as well [23]. One should consider that these novel technologies may also be exploited for cardiac use. For example, a patient could have a magnetic pill guided endovascularly to the heart and

anchored to the endocardial surface where therapeutic (biologics or drugs) agents released. Furthermore, one could even envision that the administration of these therapeutics could be controlled wirelessly and facilitated by micropumps and valves.

In the near future, it is considered that drug eluting microfabricated devices with incorporated biosensors could be implanted locally such that they release drugs in response to given physiological stimuli. For example, a small sphere, capsule, or micelle containing insulin producing cells could be implanted in the pancreas, subdermally, or intramuscularly. The cells could then respond to fluctuations in glucose levels automatically. Extensive research has gone into such closed loop systems for insulin delivery for diabetes patients, with the eventual goal of an artificial pancreas [24–27]. In the more distant future, genetically engineered cells could be programmed to produce other drugs as needed and subsequently deliver them at the proper rates or in response to particular stimuli. Furthermore, unlike implantable devices, such as drug eluting stents that can only deliver drugs for a few years, these cell-based drug producing devices have the potential to last a lifetime.

It is generally considered that delivering therapeutic agents at or near the entrance to coronary arteries would be particularly useful in certain clinical situations. Such a delivery methods could then exploit the natural capillary system to perfuse the drug to the entire heart. This could in turn potentially reduce the amounts of drugs needed significantly and may also ameliorate undesired side-effects known for systemic administrations. In another approach, during surgical operations deployed degradable microcapsules with tuned drug release profiles could be injected into the myocardium

adjacent to the coronary arteries. Alternatively, resorbable patches could be adhered

over the main coronary arteries and the therapeutic agent would then diffuse into the

vessel and be transported to the entire organ. In addition, a delivery patch could also be

adhered to local areas, such as one doped with angiogenic treatment placed on an

infarct zone.

In the future, combinatorial therapies could also be extended beyond current clinical

practice such as drug eluting stents and pacing leads. This is an area of great

opportunity for local drug delivery advancements. For example, ventricular assist

devices could incorporate the ability to passively or actively deliver a variety of therapies.

As such, their possible approaches and advantages may improve the use of these

devices, e.g., when they are being used as a bridge to transplant or recovery.

It should be noted that the direct injections of drugs, proteins or cells into the

myocardium have also been proposed as a method of local delivery [28–31]. More

specifically, these could be localized in areas of infarct or near atherosclerotic plaque

deposits to aid in restoration of normal function. To date, it is noteworthy that positive

results have been observed with injections of adenoviruses encoding for heat shock

protein [28] or growth hormone [29], [30] in rabbit and rat models, respectively.

Furthermore, clinically, gene transfer by direct injection of plasmid DNA coding vascular

endothelial growth factor (VEGF) into ischemic myocardium has promoted angiogenesis

[31].

It should be described for completeness that transmyocardial laser revascularization has been applied clinically to patients with inoperable coronary artery disease and often applied in conjunction with CABG. This procedure utilizes a laser to perforate the walls of the heart in areas of poor perfusion. The channels created act as conduit for blood and during healing neovascularization was also considered to improve perfusion. Perhaps this approach, if considered an option, could be supplemented with adjuvant use of local therapeutic agents to quicken the vascularization process, such as VEGF, or other therapeutics to potentially restore normal function.

Alternative local delivery methods

To date, numerous drug pumps and other devices and methods for delivering treatment to a localized area or region of the body have been shown to be successful. As mentioned previously there is a considered difference between a local delivery and a targeted delivery of treatments. Additionally, it is also possible that various therapeutic approaches incorporate one or both of these methods, in order to maximize the beneficial effects of the therapy and minimize the adverse side-effects.

One such method, which has been studied significantly, is to create a polymer or biological scaffold in which the drug/protein has been imbedded. Subsequent release is then dependent upon either degradation of the scaffold or diffusion of the drug out of the scaffold. Currently, there are such clinical devices available, such as the Gliadel® wafer, which is impregnated with a chemotherapy drug and used following surgical resection of cancerous tissue within the brain [32], [33]. Note that these drug delivery platforms have been made from a number of different polymers, synthetic or natural. Ultimately it is

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considered that whichever material is used, it must be biocompatible and able to dispense the drug at appropriate rates for the desired particular treatment. Currently, such products have been developed relative to the treatments for cancers, which have included polymer structures for subcutaneous or intramuscular placements. More recently, similar scaffolds have been created for the treatment of cardiac diseases, but these are still within the research phase of the design.

Myocardial patches, often made of a gel, collagen or other biocompatible material, are typically impregnated with stem cells or protein growth factors meant to diffuse into the heart to promote myocardial growth and revascularization. These are placed locally on infarcted areas of the heart. Alternatively, the scaffolds could be designed to promote growth within their structure, becoming a functioning part of the myocardium. If the device/scaffold is made such that it requires stem cell infiltration in an *in vitro* setting, it can be incubated with the particular cells needed prior to implantation onto the cardiac structure [34]. Both of these methods illustrate how tissue engineering approaches could be utilized to locally deliver drugs or therapies to the heart, however there are other mechanical devices that also allow a physician to deliver drugs directly to the site of interest.

Localized Injections and Drug Pumps

When discussing localized treatment of tissue, a method to deliver the treatment to a specific region of interest is essential. Various methods have been reported in the literature from simplistic methods of direct injections of the drug to the localized area to be treated, to the use of more complex microelectromechanical systems (MEMS). The

method for direct injection of a treatment into a specific diseased area is a fairly simplistic idea; however, the means to deliver a needle and treatment to the heart may pose a significant challenge. Current research is investigating injecting into the myocardium via angioplasty balloons to reduce the occurrences of restenosis. More specifically, small pores or openings within a catheter deliver gene therapy or alternative drug treatments to the diseased cardiac cells [35], [36]. Likewise a given drug can be coated directly onto the exterior surfaces of a balloon, i.e., when the angioplasty is performed the coating rubs off on the wall of the vessel to provide therapy. Recently, Scheller and colleagues demonstrated that such a device was able to decrease the incidence of restenosis significantly [37]. Stents themselves can also be thought of as a method for localized delivery of a drug to a site specific location. This approach has been well developed and tested: drugs like paclitaxel have been coated onto the outside of a coronary stent so to minimize restenosis that can occur at sites of the stent implantation [38].

Other treatments that might be given to a patient may need to be localized but cover a greater area than a single location along an artery or vein. For those purposes, devices such as MEMS or osmotic pumps could perhaps be utilized to slowly delivery treatment to a specific site continuously or intermittently and with varying rates; i.e., delivery could last from days to months. When considering drug pumps, they generally fall into one of two categories, passive or active. The passive pump approach can be thought of as being similar to the gels or wafers discussed previously; however instead of the drug being imbedded within the polymer, it would be within a chamber that would be opened once the polymer had been degraded enough to release it. To date, multiple designs

have been implemented including multiple wells in a row with differing polymer closures to release at different times, as well as multiple chambers to release two different types of drugs simultaneously. As one could imagine the only limitations to these types of designs are the properties of the polymers and the size of the device [32], [39].

One specific design described for a passive system is fairly unique, is the use of osmotic pressure to push out the drug from an almost syringe like device. These work on the principle that water will diffuse one way across a semipermeable membrane and increase the pressure on one side and thereby pushing out the drug slowly [39]. These types of devices have also been modified slightly to create pulsatile drug delivery pumps, i.e., in one case, a membrane was setup and had immobilized glucose oxidase which converted glucose to gluconic acid [40]. This causes an ionic change in the membrane and the electrostatic repulsion of the membrane causes an expansion and increased delivery of insulin. This has the added benefit of the ability to adapt to the specific needs of the patient at various times of the day, depending upon glucose and levels within their system [40].

Another type of the MEMS approach for agent delivery is to release a bolus from a small reservoir; initially these devices relied on an electrochemical dissolution of a gold membrane blocking a reservoir of a solution. However, a number of published studies to date have reported that this approach could not be performed reliably, so it was modified to a localized melting of the gold membranes by resistive heating [32]. Another approach of an active delivery pump has been employed for patients with chronic pain, e.g., a pump with a reservoir filled with a pain medication can be implanted with a catheter

leading directly into the spinal column or other neuronal targets. It should be noted that more recently the ability to refill these devices has been greatly improved and this is an advantage over MEMS devices that cannot refill, however the size of the former devices are currently much greater [41]. Nevertheless, both of these types of pump systems have their own specific purposes, yet both intend to deliver a drug treatment to a localized area within the body to help reduce the effects of the drug on other organs or nearby healthy tissue.

Ultrasound and Lipisomal Delivery Approaches

Another delivery technique is encapsulating agents for release in specific areas. As opposed to the local delivery pumps described above, where a drug is released into a specific location within the body, generally these packaged therapies can be administered intravenously with targeted release. In these cases, the drug will circulate throughout the entire body, but importantly the targeted drugs have been altered in a way to make them either: 1) released at a specific site, 2) preferentially bind within the diseased area, 3) be more readily taken up by the target cells, 4) preferentially released slowly over time or 5) any combination of these attributes. Ultimately the aim is to increase the effectiveness of the treatment whilst decreasing the toxic systemic effects of the drug. These approaches can be considered compatible with localized delivery, especially in the setting of thoracic surgery. For instance, a targeted drug could be infused into the coronary arteries, compounding the targeted design with local delivery.

Another specific way that investigators have been able to achieve the targeted delivery is by using liposomes that encapsulate the drug, relying on active or passive targeting of tissues to be treated. First, it can act passively, relying on various cells to uptake the liposomes, e.g., reticuloendothelial cells, which can be found concentrated within the liver and spleen. Thus if the treatment was designed specifically for cells within the liver or spleen, a passive approach might be found to be quite acceptable. It should be noted that this same approach has been discussed within nanoparticle delivery as well, taking advantage of the fact that the vasculature within tumors can be porous, allowing the nanoparticles to accumulate within the tumor region. It is still important, however, to aim to limit peripheral exposures, since such liposomes may still be taken-up within the liver and other filtering organs [39], [42].

More specifically an active targeting paradigm could be achieved by placing a recognition sequence on the outside of the liposome, so to develop ligand-receptor interactions that will in turn bind the carrier to the targeted cells. As such, these could be targeted to specific proteins or to biomarkers that are upregulated in specific tissues, like tumors or portions of the myocardium responding to ischemia or heart failure [2], [3]. One needs to consider that these modified liposomes may be still taken up by the liver, spleen or other non-targets; however, some specified modifications of the developed lipid layers may minimize uptake in these structures [42].

Another reported means that treatments may be delivered to target specific cells is via microbubbles aided by ultrasound disruption. More specifically, the microbubbles can be formed by air or other types of gas and introduced into the bloodstream similar to those

techniques utilized in ultrasound imaging with contrast. Note that air bubbles are more readily dissolved into the blood following introduction into the venous system, giving them a shorter lifespan. By using perfluorocarbon gases the lifespan of the delivery bubbles increase and they can also be coated with a variety of materials including polymers, lipids or proteins which will also increase the lifespan even further. These long lasting microbubbles can then be disrupted by focused ultrasound—a trigger that can be applied nearly anywhere in the body [4–6]. It is considered that the ballistics of the cavitation not only disrupts the integrity of the bubble, but it will also momentarily disrupt the plasma membranes of the target cells, allowing for the drug and/or microbubbles to be passed through [43], [44]. While that this approach has resulted in detrimental effects on cardiac mechanical function [45], it has been shown to effectively increases absorption of certain drugs [46]. Alternatively, the capsules can be designed to release their therapeutic payload with a slight increase in temperature that can be triggered by local heating [47].

Pericardial delivery

It has been noted that the pericardium provides a unique space that holds a vast potential for localized drug delivery. For example, such pericardial approaches may range from: 1)the delivery of preconditioning therapies during surgical preparation, 2) providing for therapies that promote vascular genesis after CABG, and 3) the prophylactic administration of antiarrhythmic agents in order to prevent postoperative AF. We consider here the possible treatments for the myocardium are numerous and will provide a few specific examples below, but first we will review one's pericardial anatomy.

Anatomy of the pericardium.

The pericardium is made up of two connected structures. The innermost layer is serous membrane, which is inseparable from the epicardial surface, is called the "visceral pericardium." The continuous serous membrane is folded in on itself and the single layer also makes up the inner surface of the parietal pericardium. The single layer of mesothelial cells is indistinguishable from the fibrous outer layer (Figure 2.2). Together, the parietal and fibrous layers make up the outer layer, or "parietal pericardium." This is the most prominent layer of the pericardium and is what we generally think of when we discuss the pericardium.
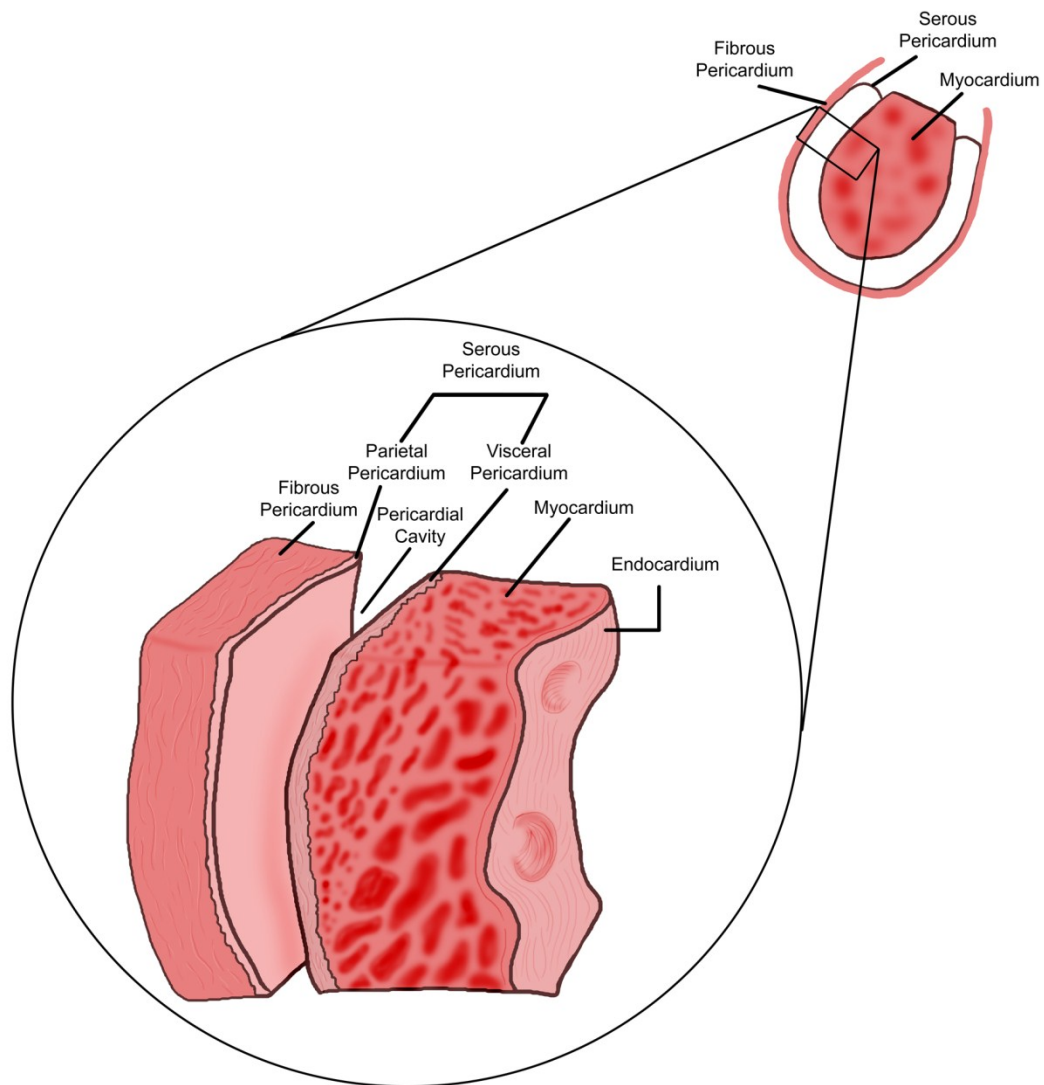
Figure 2.2: Schematic of the layers of the pericardium. The upper right shows a schematic diagram of the serous and fibrous pericardium is shown with respect to the heart. The expanded cross-section view shows the attachment of two layers of the serous pericardium (visceral and parietal) to the myocardium and fibrous pericardium, respectively.

The healthy pericardium contains between 20-60mL of pericardial fluid. This fluid, an

ultrafiltrate of the plasma [48], surrounds the heart, with the majority in the pericardial

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sinuses and atrioventricular grooves. This fluid normally drains into the lymphatic system at a relatively slow rate, measured to be a volume equivalent every 5-7 hours in sheep [49]. However, as pericardial fluid pressure increases, such as in the case of "cardiac tamponade," investigators have found that not only does lymphatic drainage increase [50], but fluid may pass through the pericardium and enter the pleural space [51].

Though the volume of pericardial fluid is not evenly distributed, it is generally found to be well mixed due to the motion of the heart; thus agents can be considered to be quickly and evenly dispersed throughout [49]. Even though there is only a relatively small amount of fluid circulating around the ventricles, this aforementioned mixing action will help maintain even distribution of any additions to the pericardial fluid epicardially, thus maintaining consistent gradients relative to the myocardium. While the parietal pericardium is generally considered as non-compliant, the overall pericardial space can accommodate moderate increases in the amount of fluid by filling in the pericardial sinuses. However, once this reserve volume space is filled, pericardial pressure quickly increases with added volume, i.e., symptomatic tamponade is elicited ().
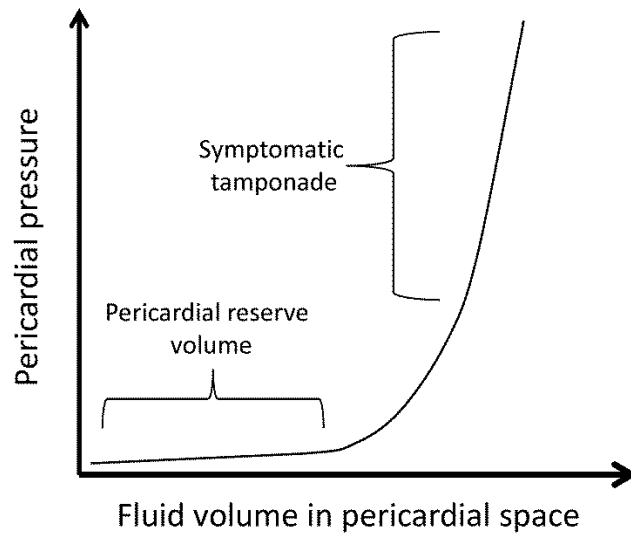
Figure 2.3: Percardial pressure vs. pericardial volume. As pericardial fluid volume increases, the pericardial reserve volume is filled. Once the reserve volume is full, pressure within the pericardium rapidly rises causing cardiac tamponade and functional depression.

The basic physiology associated with the pericardium

The fibrous (parietal) pericardium is 1-3 mm thick in healthy humans, and as note above it is considered as minimally or non-compliant. In fact, because of these features, multiple bioartificial replacement heart valves are made with leaflets of either bovine of swine pericardium. As such, this tough layer has the primary function to physically constrain the heart. While this may not have a large influence at rest, during physical exertion, cardiac filling becomes limited by the pericardium. Further, it has been noted that an intact pericardium also increases cardiac chamber interdependence, i.e., increased pressure in one chamber affects other chambers because the total volume is restricted by the pericardium. A more detailed review can be found in the Handbook of Cardiac Anatomy and Devices [52].

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Typically during cardiothoracic surgery, the pericardium needs to be opened so to obtain direct myocardial access and at the end of a procedure, the pericardium is not typically closed. This in turn reduces the risk of post-surgical cardiac tamponade, as pressure cannot build up easily in an open pericardium. However, the lack of a barrier between the heart and the healing incision site typically leads to scaring and epicardial fusions within this wound site. Typically, this has minor consequences—that is until a subsequent open heart procedure needs to be performed, and both the initial incisions and heart access are complicated by these additional fibroses. It has been suggested that a barrier placed between the sternum and myocardium (with or without bypass grafts), would potentially limit the buildup of subsequent adhesions and make reentry less risky. While synthetic barriers such as the absorbable CovaCard (BIOM'UP, Lyon, France) are being developed [53], the native or graft pericardium also may provide a natural and available option.

Relative to physiological consequences, in addition to reducing reoperative complications, the closure of the pericardial sac following cardiac surgery has been proposed to reduce long term cardiac performance and aid to maintain diastolic functions and ventricular geometries, as well as reduce right ventricular dysfunctions [54], [55]. Additionally, one could also consider that a closed pericardium may also provide a reservoir space for subsequent pericardial therapies. Yet, one reported limitation to pericardial closure is that it can acutely reduce cardiac indices and stroke

work [56]. More specifically, Rao et al. corroborated that these functions were reduced

one hour postoperatively in patients who had pericardial closure (P<0.001). However,

they also reported no significant differences in functions between patient groups at 4h or

8h postoperatively. While increased risk of cardiac tamponade still exists with full

closure, it has been reported that fenestrated techniques and pericardial drainage tubes

have been used to mitigate the consequences of pericardial effusions [57], [58].

In summary the pericardial space potentially provides a natural barrier and is well suited

for localized drug delivery. Thus if pericardial access could be easily and reproducibly

obtained, the possibilities of long- and short-term treatments include: antiarrhythmic

therapies, the delivery of agents to reduce cellular injuries at reperfusion and/or the uses

of angiogenic proteins to promote revascularization and regrowth specifically within

infracted regions.

Cardioprotective agents

To date within the US, ischemic heart disease and myocardial infarctions remain as

leading causes of clinical morbidities and mortalities. Their occurrences often lead to

congestive heart failure, which in turn causes further reductions in coronary flows and

the necrosis in the myocardium and leads to further functional impairments. It is

generally considered that compared to other body tissues, myocardial cells have poor

regenerative abilities: a fact that has focused much research on methods for reducing

trauma and/or myocardial death, as well as methods for improving repair and regeneration.

It has been reported that the local infusion of nitric oxide donors could promote local vasodilation without major systemic effects [59]. Additionally, the administration of vascular endothelial growth factor (VEGF) and other angiogenic agents into the myocardium have been associated with several benefits that include increased collateral vessel developments, increases in regional myocardial blood flow, improved myocardial functions in the ischemic regions, and/or increased myocardial vascularity [31], [60–62].

Relative to such, our laboratory has observed that intrapericardial delivery of omega-3 polyunsaturated fatty acids can dramatically reduce both infarct sizes and ventricular arrhythmias associated with ischemia. More specifically in this study, acute ischemia was induced for 45 minutes followed by 180 minutes of reperfusion while the omega-3 fatty acid DHA was delivered to the pericardial space prior to ischemia as well as the initial period of reperfusion. Importantly during the ischemic period, ventricular arrhythmias were reduced 50% (which in the control hearts require defibrillation and cause 20% mortality). Upon completion of the reperfusion, the hearts were excised and ischemic damage was measured: hearts treated with DHA had a similar area at risk, but an 57% reduction in normalized infarct size was seen [63]. Ongoing research in our lab also suggests that omega-3 fatty acids may also reduce susceptibilities to AF during cardiac surgery.

Most recently, investigations in our laboratory have dealt with the pericardial delivery of specified bile acids noted to have anti-apoptotic benefits. These molecules are upregulated within hibernating black bears and reports by others have suggested that ursodeoxycholic acid may be beneficial in reducing AF within myocytes [64]. In these ongoing studies to determine the potential beneficial effects within a large animal model, we specifically deliver a taurine conjugate of ursodeoxycholic acid within a formed pericardial cradle (the pericardial space) and periodically induce AF. Preliminary results have suggested that these molecules are effective in reducing the times a given heart will elicit AF: i.e., without having to give this therapy intravenously and thus potentially have undesirable systemic side effects (unpublished data).

Antiarrhythmic agents

Antiarrhythmic drugs are commonly known for their narrow therapeutic ranges and severe side-effects. It has been previously suggested that delivery of these agents to the pericardial space would allow for myocardial diffusion while lowering undesired plasma drug concentrations [65], [66]. In other words, such a local delivery allows for higher doses of this class of agents to be safely administered to control focally the heart rhythm—an application that could be especially applied during cardiothoracic surgery. To date, the intrapericardial delivery of antiarrhythmic agents has been attempted with numerous agents, e.g., esmolol [65], solatol [67], atenolol [67], ibutalide [68], procainamide [69], [70], digoxin [69], amiodarone [71], arachadonic acid [72], nitroglycerin [59] and L-arginine [73] have all been shown to have electrophysiological

effects when delivered to the pericardial space in various animal models. Additionally, in those studies that also measured plasma concentrations, they showed minimal crossover of the delivered agent into the bloodstream [69–71]

To date, despite these reported successes in treating arrhythmias in various animal models, the clinical practice of intrapericardial (IP) delivery is not widely employed. Possible reasons include:1) the lack of experience (no large clinical trials), 2) difficulties with access and removal of agents and/or 3) unknown potential complications with this delivery route. It is important to note that one of the described major concerns with pericardial delivery is that it relies primarily on trans-epicardial diffusion to reach the myocardium. In other words, while the thin atria and superficial SA node may be easy to treat via these mechanisms, the effects of possible ventricular drug gradients are not well defined. Further, it has been hypothesized that moderately soluble or lipophilic molecules will not be evenly transported across the thicker ventricles, causing various degrees of electrophysilogical changes through the depth of the myocardium, creating a scenario where the epicardium and endocardium are not conducting and/or contracting at similar rates. This electrical heterogeneity theoretically carries the possibility of initiating, rather than inhibiting, arrhythmias [70].

In our lab, we have investigated the pharmacokinetics and pharmacodynamics of IV and IP delivery of metoprolol [7]. While the β-blocker is typically used to treat angina and hypertension, it is also used to treat tachycardias. With a tachycardic pig model, IV delivery of metoprolol was faster acting compared to IP, but only by several minutes. While the reductions in heart rates were similar for both, these effects were sustained

longer after IP delivery. Importantly, IV delivery was accompanied by significant

reductions in contractility, while IP delivery elicited minimal effects. In other words, these

findings indicated that IP deliveries of metoprolol may have similar bradycardic effects

compared to IV deliveries, but without the reduced contractility. The other important

finding in this study was the minimal pericardial crossover of metoprolol within blood, as

well as the slightly increased half-life of the drug [7].

Clinical Pericardial Access

Access to the pericardial space for the delivery of therapies, outside of cardiothoracic

surgical procedures, may pose many difficulties. However, multiple minimally invasive

procedural methodologies are under development. For example in the trans-atrial

approach, access to the pericardium is achieved via a catheter coming up through the

femoral vein into the right atrial appendage, where it then punctures through this thin

myocardium to gain access into the pericardial space. To date, success with this

approach has been demonstrated in canines and swine [74]. Alternatively, a subxyphoid

access procedure has been suggested [75]. While this method is often used to drain the

pericardial space during episodes of cardiac tamponade, the minimal separation

between the fibrous pericardium and the epicardium make this approach more difficult

when there is not a substantial amount of intrapericardial fluid present. Several access

tools have and continue to be developed to aid in subxyphoid access using minimally

invasive access. For instance, the PeriPort™ (Cormedics, TX) system is designed so to

initially enter the thoracic space through a subxyphoid incision where it uses a vacuum

on the distal end to grip the pericardium and separate it from the heart's epicardial

surface. Once the pericardium is pulled into the vacuum chamber, a retractable needle

pierces the pericardium and allows for a near tangential entrance of a guidewire. Such

designed access tools should enhance both the safety and simplicity of pericardial

access and also have potential to increase the widespread use of localized therapies to

treat pericardial and cardiac diseases. Nevertheless, during cardiothoracic surgery, a

simple syringe or perfusion pump is all that is clinically needed to delivery pericardial

therapies. Thus the hurdles to add localized pericardial delivery of drugs concurrent to

surgery are much less compared to pericardial therapies on their own.
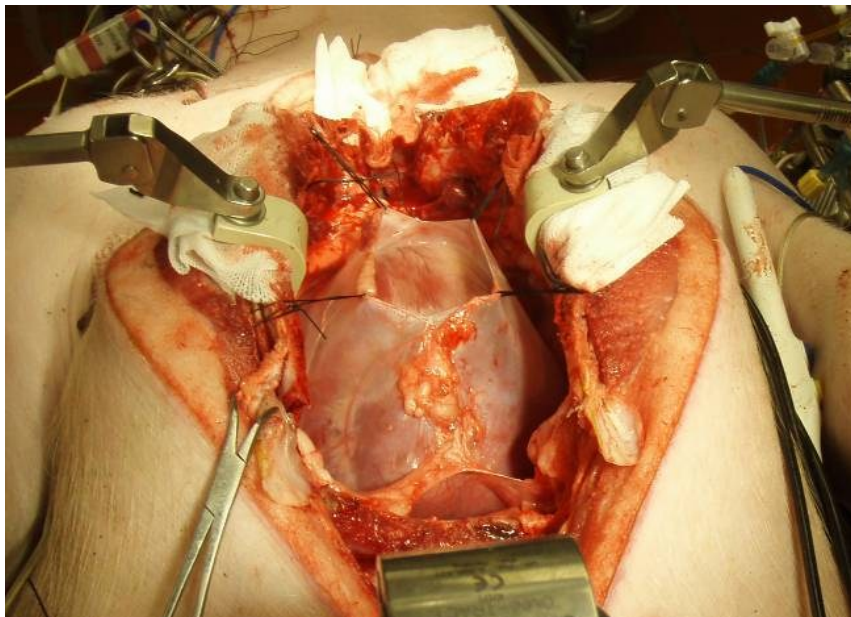
Figure 2.4: Methods of pericardial access include the pericardium as a reservoir. Pictured here is a pericardial cradle in a swine model.

Protocols

As noted above, the pericardial delivery of molecules, cells, nanoparticles, etc, becomes

simplified with surgical access (Figure 2.4). Without the requirement of added

procedures and incisions, the pericardial delivery of therapeutics can be administered

with minimal additional equipment, time or complications, leading to numerous clinical scenarios that may benefit from for the use of pericardial delivery. While some of these applications, as described below, have gone through pilot studies in animals, future clinical studies have yet to be developed to confirm efficacy.

In one such application, a common first choice of donor vessel for a CABG procedure is the internal thoracic artery. Thus, while the surgeon initially frees and prepares this vessel for subsequent grafting, the pericardial administration of either a prophylactic antiarrhythmic or angiogenic agents could be infused into the pericardium. Once the artery has been prepared and the pericardium is further opened, the drug will have had a chance to partially diffuse into the myocardial tissue. Additional drug could also be added through direct myocardial injection to promote revascularization or prevent postoperative arrhythmias. Alternatively, a procedure that requires access from the anterior surface of the heart may make use of the reservoir created by the remaining pericardium to treat the posterior myocardium during the procedure.

Another important approach to consider is the postoperative administration of drugs, which could be accomplished via surgically placed transdermal drainage catheters. More specifically, with the placement of drainage catheters and temporary pacing leads at the end of a procedure, the addition of a catheter leading into the pericardium is sometimes included [76]. This catheter, whether through the main lumen or a second lumen, could hypothetically allow for continued access to the pericardial space and

delivery of appropriate antiarrhythmic, antibiotic, or other therapies to the myocardium. Therapies with such devices have been attempted with success in a porcine model [77].

As mentioned above, multiple techniques exist for low or no tension pericardial closures [58], [78], [79]. In addition to the reoperative benefits, the planned closure of the pericardium can create a useful reservoir for localized treatment. While the most common clinical reason for non-closure is to reduce the risk of cardiac tamponade, this could be mitigated with the placement of the aforementioned specially designed drainage/therapy delivery catheter. We also believe that with full pericardial closure and access via the pericardial drain, a delivered therapy would reach all surfaces of the heart.

Another clinical situation/option for localized drug delivery might be during cardiopulmonary bypass procedures. To arrest the heart and often throughout the procedure, cardioplegia solutions are perfused through the coronary vessels. Whether antigrade or retrograde, this perfused solution cools and protects the heart by minimizing metabolism. It has been considered that myocardial damage can be further reduced by introducing cardioprotective agents via this delivery route [80–82]. It is important to note that in this unique situation, any administered drug reaches the entire myocardium via the capillaries, but will have no access to other tissues until after cross clamping is released. In other words, high transmural and widespread myocardial concentrations can be achieved with remarkable speed and accuracy while minimizing side effects.

Similarly, organ transplantation offers ultimate accessibility in localized drug delivery. A typical transplant includes explant, transportation and re-implantation. Because of the pre-explant recovery of additional organs, localized delivery is a viable option where the effects can be greatly enhanced post-re-implantation function. Not only are options such as pericardial delivery still available, since the heart is typically the last organ removed, IV therapies just prior to cross-clamping become targeted.

Next, the times between explant and implant can range from under six hours for hearts to up to 24 for the liver or kidney. During these times, the heart is often in stagnant cold storage. However, ideas and methods for continuous perfusion during the hypothermic period are almost as old has heart transplantation itself, e.g., continuous hypothermic perfusions (CHP) of the heart was first applied only a year after the first successful human heart transplant [83]. Since then it has been shown that it is possible to keep large mammalian hearts viable for up to 48 hours in baboons and swine [84], as well as improving functions compared to non-perfused hearts [85]. While CHP is not used clinically with heart transplant, it should be noted that the Organ Care System (Transmedics, Andover, MA) gained investigational device exemption status in 2007, and a clinical trial is currently underway. This continuous perfusion during transport before implant provides another opportunity for localized therapies, as they could be simply added to the perfusates.

Potential limitations of local and target deliveries
Localized delivery methods such as those into the pericardial space are not without their potential limitations. Without direct access to the bloodstream, an administered agent at a target tissue such as in the pericardial space must diffuse into the target tissue to

increase concentrations. While this may ultimately limit the depths or distances a given

therapeutic agent (drugs, cells, nanoparticles, etc.) might be able to migrate in a

significant quantity, it is also one of the major advantages of localized therapies.

Some areas for localized treatments, especially within the myocardium, may be

considered as relatively difficult to access. However, with a large number of cardiac

surgeries currently being performed and an increasing number of catheter procedures

being developed and implemented, these discussed therapeutic methods could

piggyback on these procedures with few added complications or risks. Finally, novel

therapeutic deliveries, drugs or other interventions may need to go through additional

approval in order to gain indication for localized delivery.

Conclusions

Cardiothoracic surgeries in themselves may allow for novel opportunities for local drug

delivery, such as overcoming the hurdle to obtaining pericardial access. Such clinical

procedures also provide opportunities for localized injections or coronary infusions. The

future opportunities for such therapies are not limited to the duration of the operation: by

leaving a pericardial drainage catheter with delivery features, physicians could also

incorporate subsequent therapeutic delivery. In some cases, implantable drug pumps or

biodegradable patches could provide therapy to the patient beyond their ICU stay. The

emerging field of localized therapy delivery shows great potential, but future human

studies are needed to verify the positive results observed in pre-clinical studies.

Nevertheless, the unique access afforded by cardiothoracic procedures may speed up

implementations of these promising local and target therapeutic delivery methods, thus

placing surgeons at the cutting edge of these novel delivery approaches.

Chapter 3: Cardiac Responses to the Intrapericardial Delivery of Metoprolol: targeted delivery compared to intravenous administration

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Preface

In this chapter, I focus further on pericardial delivery, comparing pharmacodynamics and pharmacokinetics of intrapericardial (IP) delivery compared to the more conventional intravenous (IV) delivery. While several surgical and minimally invasive methods were attempted in preparation for this study—including percutaneous and subxyphoid access—the most reliable and repeatable model we developed takes advantage of the pericardial access obtained during surgery. This open chest model not only provides full access to the pericardial space, but also allows for easy epicardial lead placement for electrophysiologic monitoring.

In order to develop an effective model, numerous stimulation protocols were attempted to induce AF. The two second burst pace at 50Hz proved to be most effective. This burst pace put all animals into AF for at least a few seconds, with about 10% if the animals maintaining AF for more than one minute, which we defined as "sustained." When this stimulation is repeated several times, almost 50% of animals had a sustained episode, giving a comparison point if a treatment could reduce total AF.

In order to perform this study, an acute model of sinus tachycardia was developed. Since a physical method such as a hemorrhagic model was deemed too risky, a pharmacological method was used. Epinephrine effectively raised heart rates, but the excessive pressures made such a model risky as well. Isoproterenol, however, proved to be a much more sustainable agent. Using this beta-agonist, it was possible to titrate the heart rate to just above 180bpm for 60-90 minutes during the experiment. This chapter has been published in the Journal of Cardiovascular Translational Research.

Summary

Anti-arrhythmic drugs have narrow therapeutic ranges and typically can engender harmful side-effects. The intrapericardial (IP) delivery of anti-arrhythmic agents proposes to achieve higher myocardial levels while minimizing plasma concentrations, thus diminishing systemic side-effects. Furthermore, IP delivery enables concentrations at the target site to be more precisely controlled. Our study objective was to compare the relative cardiac effects of intrapericardial administration of metoprolol to standard intravenous (IV) delivery in a swine surgical model.

In order to answer the question of how IP metoprolol affects sinus tachycardia, atrial electrophysiology, and pharmacokinetics compared to IV delivery, a medial sternotomy was performed on 21 swine that were divided into three groups. 1) After inducing sinus tachycardia, metoprolol boluses were delivered IP (n=4) or IV (n=4); 2) metoprolol was administered either IP (n=3) or IV (n=3) with saline controls (n=3), and electrophysiologic data were collected; 3) metoprolol levels were tracked both in the blood (IV, n=2) and pericardial (IP, n=2) fluid.

After either IP or IV delivery of metoprolol, heart rates were lowered significantly to 70% and 73% of control rate, respectively. The therapeutic effect of IV-administered metoprolol was considerably reduced after 1 hour, but was sustained longer in the IP group. Additionally, ventricular contractility and mean arterial pressure parameters were significantly lower in IV-treated animals, but were nearly unaffected in IP-treated animals. With IP administration, the elimination half-life of metoprolol in pericardial fluid was 14.4 minutes with negligible accumulations in the plasma, whereas with IV delivery,

the elimination half-life in plasma was 11.1 minutes with negligible amounts found in the pericardial fluid.

The targeted intrapericardial delivery of metoprolol effectively lowers heart rates for sustained periods of time, with minimal effect on either ventricular contractility or mean arterial pressure. We did not observe dramatic changes in induced atrial fibrillation times or refractory periods using this model.

Key Words: Target drug delivery; anti-arrhythmic agents; hemodynamics; elimination half-life; intravenous administration

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Introduction

Anti-arrhythmic drugs are known for both their narrow therapeutic ranges and potential harmful side-effects. In contrast, the intrapericardial (IP) delivery of anti-arrhythmic drugs has been proposed to achieve high myocardial concentrations while inducing minimal plasma concentrations [1-3], i.e., lower drug plasma concentrations would minimize systemic side-effects. IP delivery of such agents also has the benefit that concentrations at the target site can be more precisely controlled. Furthermore, several anti-arrhythmic drugs have narrow windows of systemic therapeutic concentration, such that slight variations (higher or lower) could cause adverse effects or no effects at all, respectively. Therefore with the use of either oral or intravenous delivery, drug concentrations at the target cell must be extrapolated based on several assumptions, typically producing relatively poor control of concentrations within the heart tissues. Thus IP delivery may be more desirable when precise therapeutic control is needed.

Intrapericardial delivery of anti-arrhythmic drugs has been attempted recently with some success. Esmolol [1], sotalol [2], atenolol [2], ibutilide [4], procainamide [5, 6], digoxin [5], amiodarone [7], arachadonic acid [8], nitroglycerin [9], and L-arginine [10] (a nitric oxide precursor) have been administered intrapericardially to prevent or treat arrhythmias in various animal models, all of which have been acute studies, with one exception [2]. Further, in several studies it was verified that high pericardial concentrations were achieved with minimal spillover into the plasma [4, 6, 7]. More specifically, the prevention of dangerous ventricular arrhythmias was shown with administration of nitroglycerin [9], arachadonic acid [8], or L-arginine [10]. In addition, with the use of less lipid soluble compounds [6, 7], higher concentrations in superficial structures were observed (such as

within the atria and the ventricular epicardium), but lower concentrations were detected within the ventricular endocardium. Some have exploited this concentration gradient to treat the superficial sinoatrial node with beta-blockers [1], assuming that there is little penetration into the ventricles. Such a concentration gradient associated with pericardial administration of agents could also be used to target atrial fibrillation with minimal ventricular side-effects [4, 6, 7].

Despite many reported benefits, the clinical utilization of IP delivery has yet to be widely employed, possibly due to difficulty in access, lack of experience with this method, and unknown potential complications. For example, underlying aspects of cardiac physiology may be affected by IP delivery, particularly the cardiac autonomic nervous system [8]. Perhaps the major concerns related to utilization of this approach are the unknown effects of concentration gradients that less soluble drugs will elicit transmurally across the ventricular myocardium. For example, if the electrophysiologic properties of the epicardium are affected by the pericardial target delivery of a drug but endocardium is not, this may initiate rather than inhibit arrhythmias [6].

One of the primary objectives of this study was to assess the acute effects of IP delivery of metoprolol on sinus tachycardia and compare this approach with the current clinical standard of intravenous delivery. In addition, we considered that the most likely clinical application of IP delivery would initially be in the post-operative setting, i.e., placement of an intrapericardial catheter would be trivial and the catheter could be removed before discharge from the hospital. Further, the occurrence of post-operative arrhythmias is extremely common, and intravenous metoprolol is often used in the event of post-

surgical supraventricular tachyarrhythmias. However, metoprolol has an adverse effect on cardiac contractility, which is particularly dangerous to the recovering cardiac surgical patient. In the pericardium, the moderately lipid soluble metoprolol should be able to diffuse into the thin atria and SA node, but may have lower concentrations deep in the ventricle. Thus, we hypothesize that IP (target) delivery of metoprolol would be an effective means of treating post-operative arrhythmias without affecting contractility.

Methods

Twenty-one male Yorkshire swine (70-90 kg) were sedated with intramuscular telazol (500 mg), and thiopental (500 mg) was administered via an ear vein. Each animal was then intubated and anesthesia was maintained with isoflurane (>1.2 MAC). An arterial line was placed in a branch of the femoral artery, and a 5-lead EKG was employed to continuously monitor heart rhythms. A Swan-Ganz catheter (Edwards LifeSciences, Irvine, CA) was introduced through the right external jugular vein to measure cardiac outputs, right atrial pressures, and pulmonary artery pressures. Next, a pressure catheter (Millar Instruments, Houston, TX) was introduced into the left ventricle (LV) through the right carotid artery to continuously measure LV performance. A medial sternotomy was then performed on each animal, and a small incision was made on the anterior surface of the pericardium. The heart's pericardium was then *tented* with suture to form a reservoir for drug/saline delivery, as further described by Ayers et al. [7] and shown in Figure 3.1. Animals were sorted into one of three protocol groups after stabilization. All surgical procedures were approved by the Institutional Animal Care and Use Committee of the University of Minnesota.
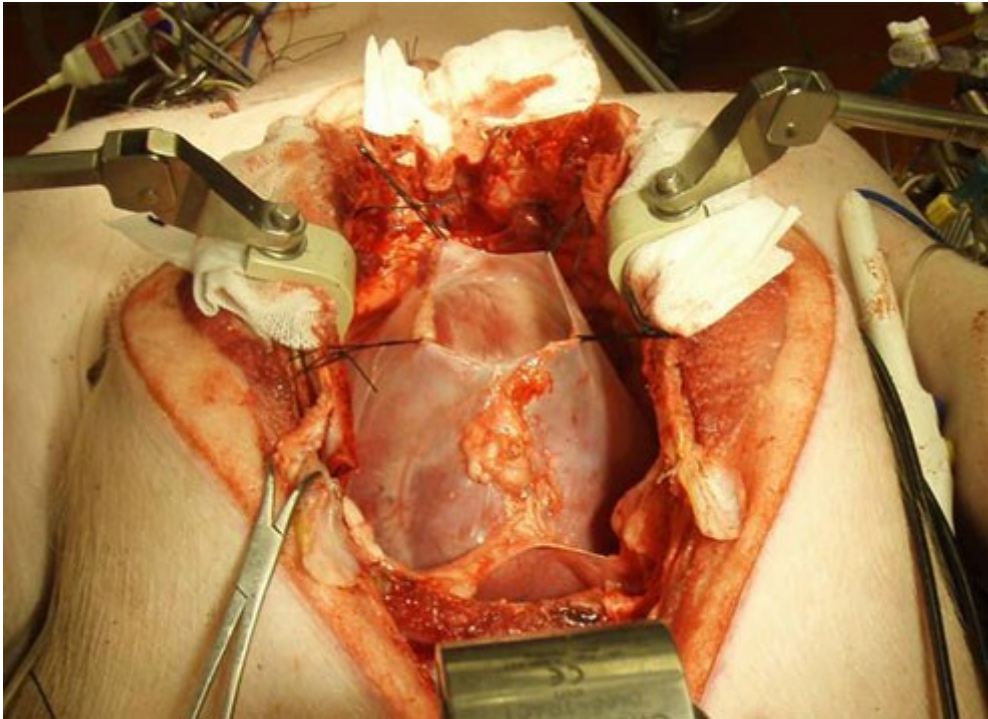
Figure 3.1: Pericardial sac tented with suture after a medial sternotomy. This tenting provided a reservoir for the metoprolol treatment, and could be easily performed during many cardiac surgeries.

Metoprolol in Sinus Tachycardia (Group 1)

Animals in Group 1 (n=8) received a slow infusion of isoproterenol (0.5-1.0 mcg/min) through the distal port of the Swan-Ganz catheter which was titrated until the heart rate was over 180 bpm; the infusion was kept constant for the remainder of the study. It was previously shown [7] that this is a reliable model of sinus tachycardia which can be sustained for hours in canine experiments. Additional electrodes were placed on the chest and limbs to approximate a full 12-lead EKG. After the heart rate stabilized, a bolus of 20 mg of metoprolol (1 mg/ml standard intravenous injection, Novartis Pharmaceuticals Corp., East Hanover, NJ) was given randomly, either intrapericardially

(n=4) or intravenously (IV; n=4). When metoprolol was given IV, 20 ml of saline was also administered into the pericardial space (sham procedure). Hemodynamic and 12-lead EKG data were taken before delivery and up to one hour after the boluses of metoprolol. Sequential samples (500 μl) were drawn from the pericardial space at -5, 5, 10, 20, 30, 45, and 60 minutes. Similarly, sequential arterial blood samples (5 ml) were obtained at -5, 2, 5, 10, 15, 20, 30, 45, and 60 minutes. High pressure liquid chromatography (HPLC) was employed to analyze the content of metoprolol in both plasma and pericardial fluid, using previously published methodologies [11].

Refractoriness and Susceptibility to Atrial Fibrillation (Group 2)

In Group 2 animals (n=9), we employed additional instrumentation to study refractoriness and relative susceptibility to atrial fibrillation. To do so, fluoroscopy was used to place endocardial pacing leads (3830 Bipolar Pacing Lead, Medtronic, Inc., Minneapolis, MN) in the high lateral right atrium and right ventricular apex. These leads were connected to a GEM III implantable defibrillator (Medtronic, Inc.), and a programmer/analyzer (Model 2090, Medtronic, Inc.) was used to control the defibrillator functional parameters during measurement of refractory periods. Epicardial pacing leads (Medtronic, Inc.) were also placed on the left atrial appendage (LAA) and the LV epicardium. Additionally, a decapolar electrophysiology catheter (Medtronic, Inc.) was placed transmurally through the LV myocardium to record activation times at different depths of the myocardium, i.e., to detect endo-epicardial activation discrepancies which could indicate whether the drug was affecting the myocardium spatially. Signals from the programmer, epicardial pacing leads, decapolar catheter, and EKG leads were all

recorded simultaneously using an ENSITE electrophysiology system (St. Jude Medical, St. Paul, MN).

After instrumentation and stabilization periods, control electrophysiology data were obtained. Atrial effective refractory periods (AERPs), ventricular effective refractory periods (VERPs), and atrioventricular nodal effective refractory periods (AVNERPs) were measured as previously described [7]. Susceptibility to atrial fibrillation was assessed by delivering a 2-sec, 50 Hz burst pace of 2-msec pulses of 20 V to the LAA lead, using a stimulator (Grass Technologies, West Warwick, RI). These stimuli were administered three times, and subsequently each time the relative durations of atrial fibrillation (AF) were measured. Note that if an episode of induced AF lasted longer than 1 minute, the heart was resynchronized (atrial defibrillation) with a 5 joule epicardial shock (Lifepak, Medtronic, Inc.). Finally, to assess relative hemodynamic stability during atrial fibrillation, a 30-sec burst pace in the LAA was used to induce AF in the animal, while hemodynamic measurements were recorded.

After the control protocol data were collected, animals received either: 1) 20 mg of metoprolol (1 mg/ml standard IV formulation) in the pericardial space (n=3); 2) 20 mg of metoprolol intravenously (n=3); or 3) a bolus of 20 ml of saline within the pericardium (n=3). Saline controls were used in this group because it was of interest to determine how the electrophysiological parameters would change over the course of the study. Both blood and pericardial samples were taken throughout this protocol as previously described. Electrophysiology parameters were assessed at 5, 30, and 60 minutes after drug delivery.

Pharmacokinetics (Group 3)

Metoprolol was given either intrapericardially (n=2) or intravenously (n=2) to the animals

in Group 3, and both blood and pericardial fluid samples were taken as previously

described.

Statistical Analyses

For measurements with respect to time, data were analyzed using two-way ANOVA with

repeated measures, and one-way ANOVA was used to determine differences between

the modes of metoprolol administration at given time points. To identify intergroup

differences, Bonferroni *post hoc* tests were employed. All data are presented as mean $\pm$

SEM; a $P < 0.05$ was considered statistically significant.

Results
Metoprolol in Sinus Tachycardia

After IP or IV delivery of metoprolol, heart rates were lowered significantly—to 70% and

73% of control rate, respectively. While the onset of action was slightly slower with IP

delivery, the effects were considerably prolonged (

Figure 3.2). It should be noted that the effects of the IV metoprolol bolus dose was

markedly reduced after 1 hour. Left ventricular contractility (as measured by dP/dt) was

significantly reduced in IV metoprolol-treated animals; contractility decreased to 73% of

the control measurement, but returned to baseline after 1 hour. In contrast, the relative

LV contractility was not significantly affected over time in IP-treated animals (Figure 3.3).

Likewise, mean arterial pressure was lowered considerably during IV delivery, but was

nearly unaffected with the IP administration (Figure 3.4). No statistically significant

differences were found between the groups relative to cardiac output, PR interval, or

QRS duration.
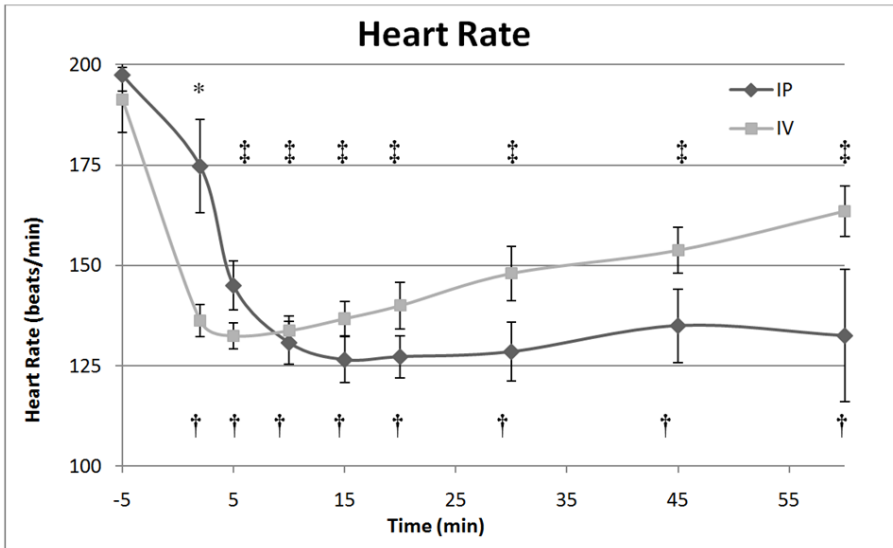
Figure 3.2: The effects of metoprolol on heart rate, delivered either intravenously (IV) or intrapericardially (IP) in a model of sinus tachycardia. The IP-delivered metoprolol took longer to induce changes in the heart rate. * significant difference between IP versus IV therapies; † IV was lower compared to -5 time point; ‡ IP was lower compared to -5 time point, $P<0.05$, n=4 for each group.
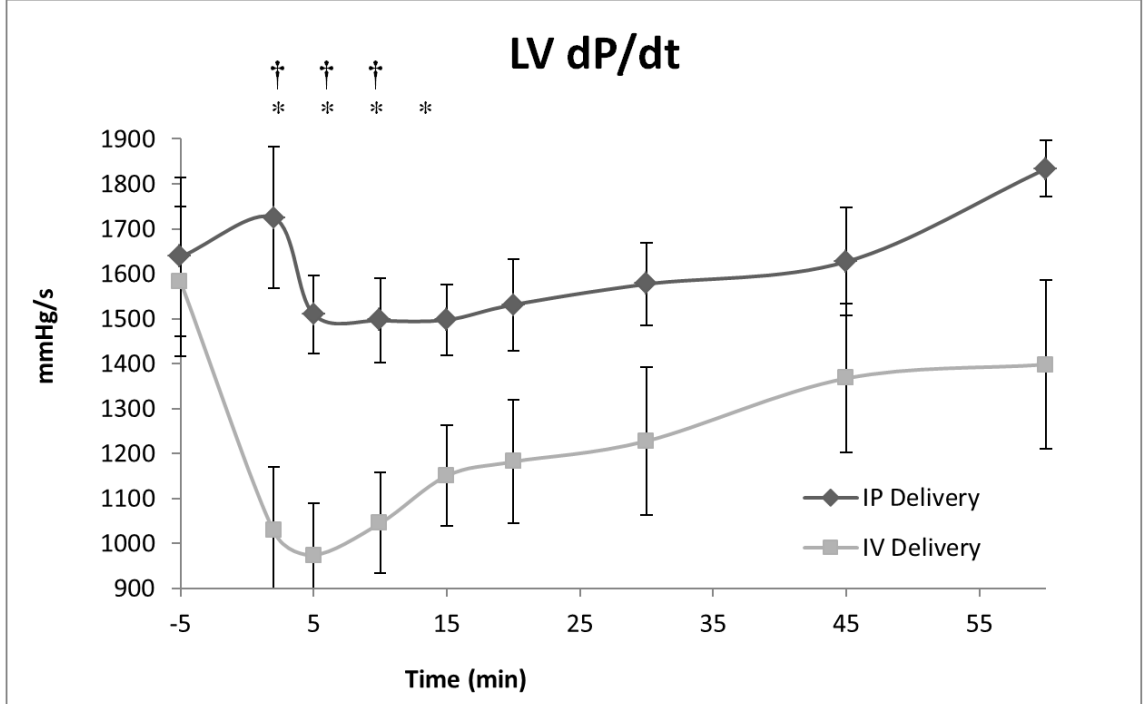
Figure 3.3: The effects of metoprolol on left ventricular contractility (LV dP/dt), administered either intrapericardially (IP) or intravenously (IV). Note that the standard clinical IV bolus delivery quickly reduced contractility for the first 15 minutes, whereas there were no significant changes in contractility with IP therapy. * significant differences between IP and IV therapies; † IV was lower compared to baseline values, P<0.05, n=4 for each group.
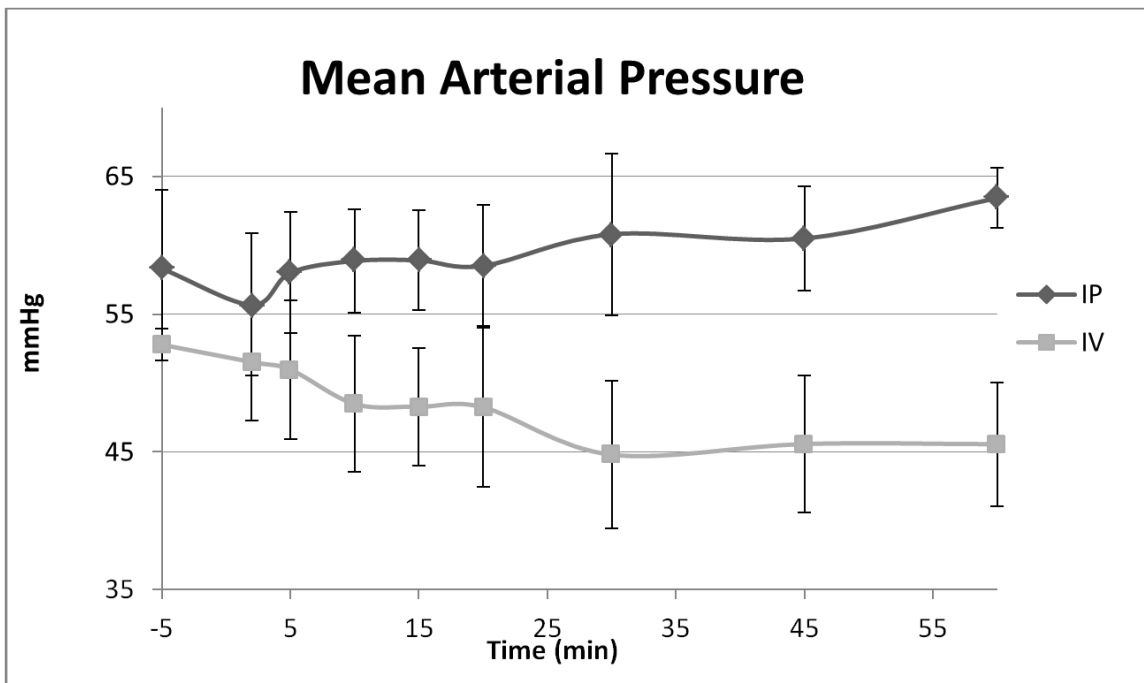
Figure 3.4: The effects of metoprolol on mean arterial pressure, delivered either intrapericardially (IP) or intravenously (IV). Note that no significant differences were observed between the two treatments or compared to the -5 minute baseline measurement (P=0.08 at t=60 min, n=4 for each group).

Refractoriness and Susceptibility to Atrial Fibrillation

The effects of therapeutic application of metoprolol on electrophysiology measurements were highly variable, thus no significant trends were observed with regards to AVNERP, VERP, or the relative ability to induce atrial fibrillation. It was considered that the porcine atrioventricular (AV) nodal physiology, and perhaps the presence of the AV node *gap*, made it difficult to measure the expected increases in AV node refractoriness with IV delivery. Furthermore, the transmural activation times, measured as the difference in activation times between the most epicardial and most endocardial electrodes, also were observed not to elicit significant changes between any of the study groups. Ventricular

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rates during AF were above 150 bpm in all animals, but no significant changes were observed ().

Ventricular rate during AF
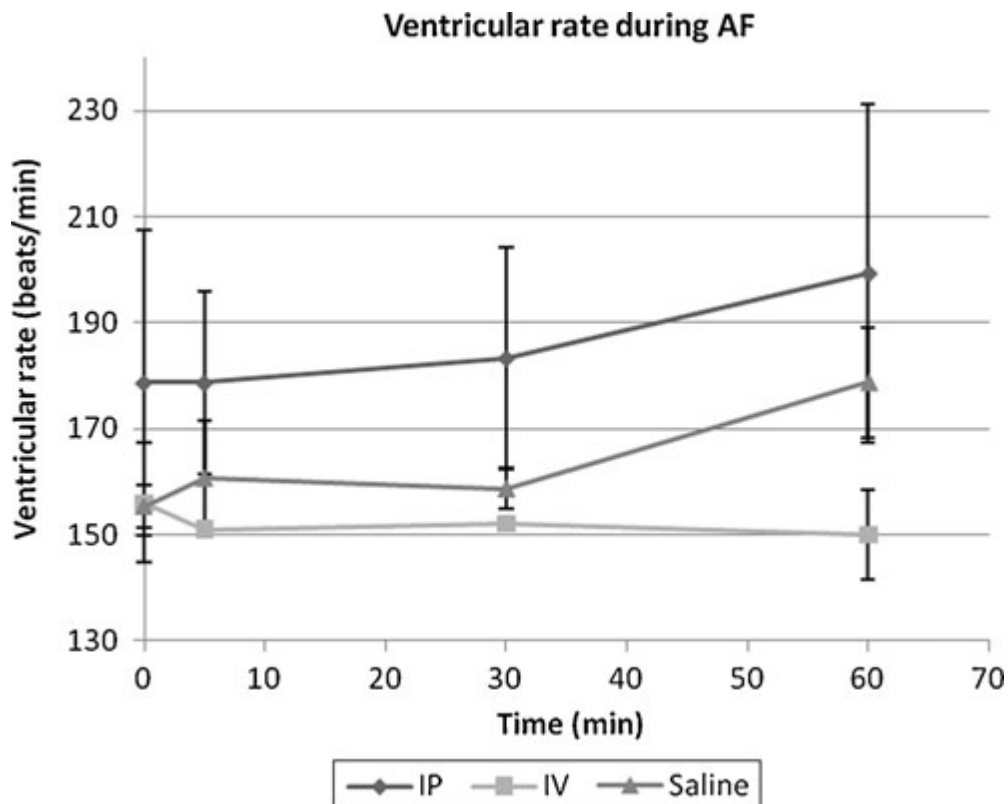
Figure 3.5: Ventricular heart rates (escape rhythms) during 30 seconds of atrial fibrillation (AF) induced via stimulation. No significant changes were observed either following the IP or IV therapies (P=0.54 at t=60 min, n=3 for each group).

Nevertheless, it should be noted that there were observed trends in the relative effects of atrial electrophysiology, i.e., inter-atrial conduction times increased in saline control animals, but remained closer to their control values in both the IV- and IP-treated animals. Similarly, AERPs decreased in saline-treated animals, but remained more stable in both IP- and IV-treated animals (and Figure 3.7).
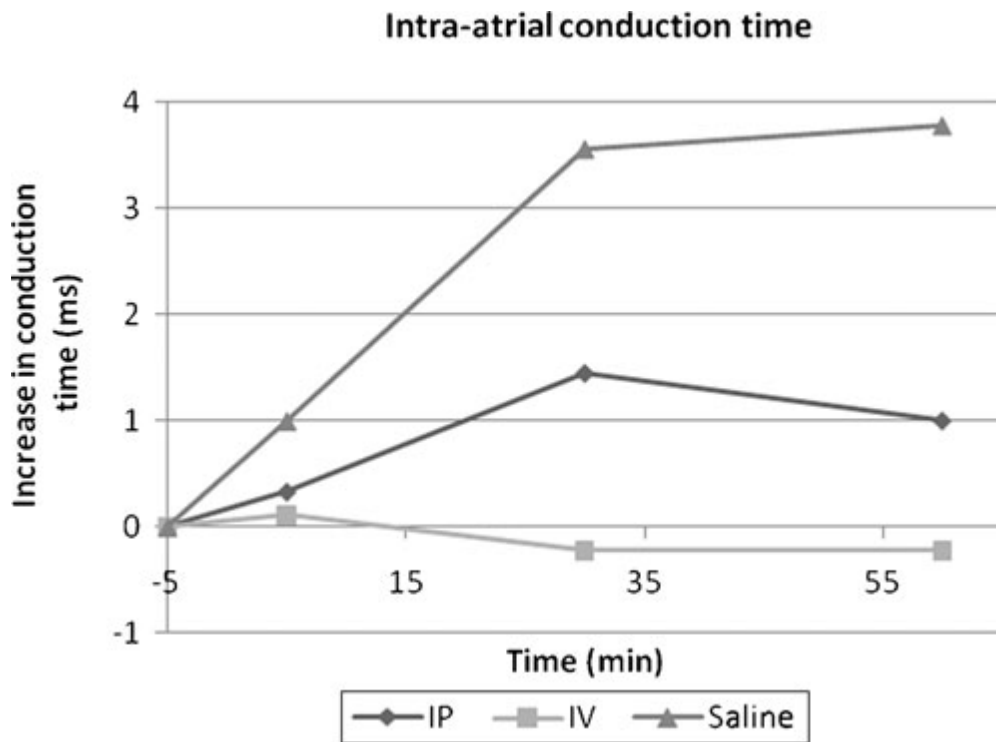
Figure 3.6: Relative changes in inter-atrial conduction times after administration of intrapericardial (IP) metoprolol, intravenous (IV) metoprolol, or IP intrapericardial saline. No significant differences were observed relative to the observed changes in conduction time (P=0.21 at t=60 min , n=3 for each group).
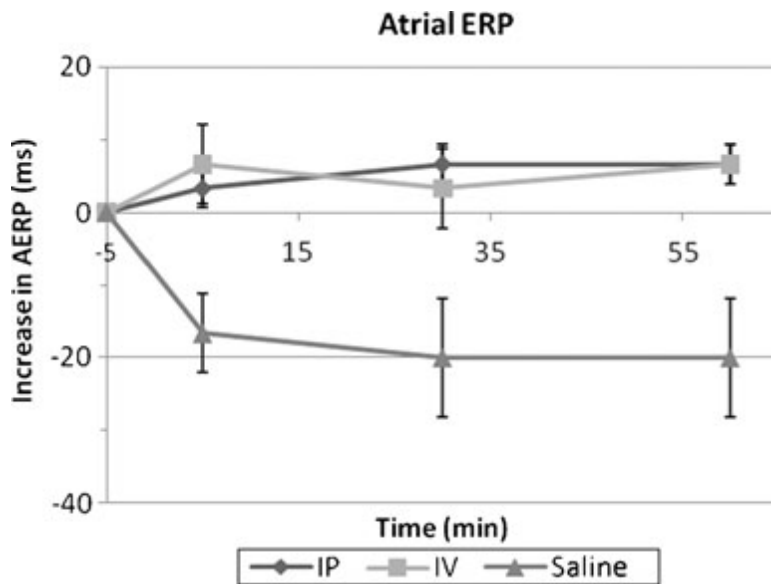
Figure 3.7: Relative changes within atrial effective refractory periods (ERPs) after intrapericardial (IP) metoprolol, intravenous (IV) metoprolol, or IP saline administration. No statistically significant differences exist amongst the treatment groups (P=0.064 at t=60 min, n=3 for each group).

Pharmacokinetics

When given intrapericardially, the elimination half-life of metoprolol in the pericardial fluid was 14.4 minutes, with negligible detectable amounts within the plasma (Figure 3.8). In contrast, when given intravenously, the elimination half-life of metoprolol in the plasma was 11.1 minutes, with negligible amounts in the pericardial fluid (Figure 3.9). It should be noted that the pericardial fluid metoprolol concentrations during IP delivery were almost 600-fold higher than plasma concentrations during IV therapy.
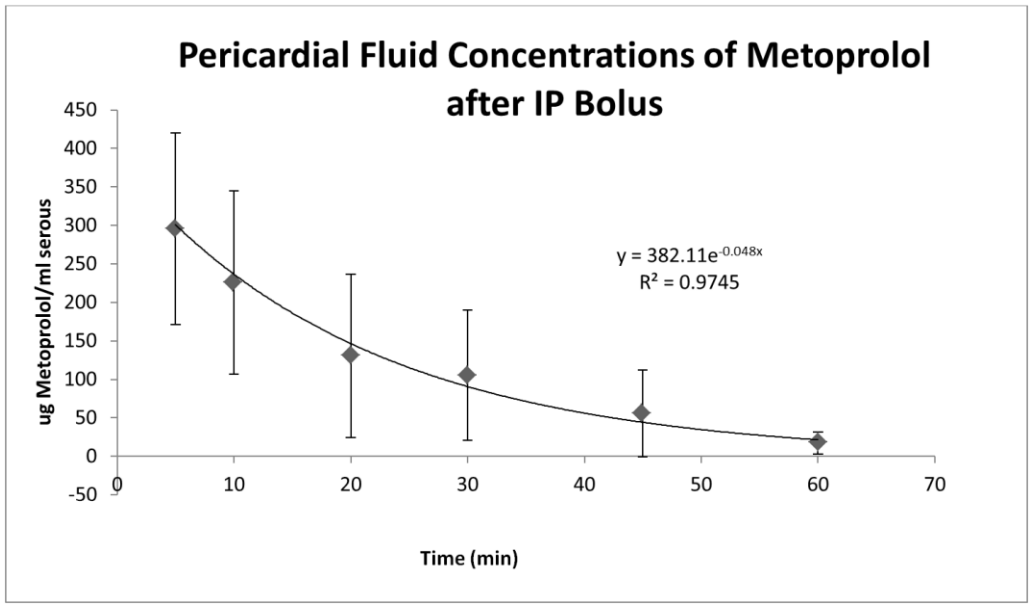
Figure 3.8: Metoprolol concentrations in pericardial fluid after intrapericardial (IP) administration; half-life was calculated to be 14.4 minutes (n=2).
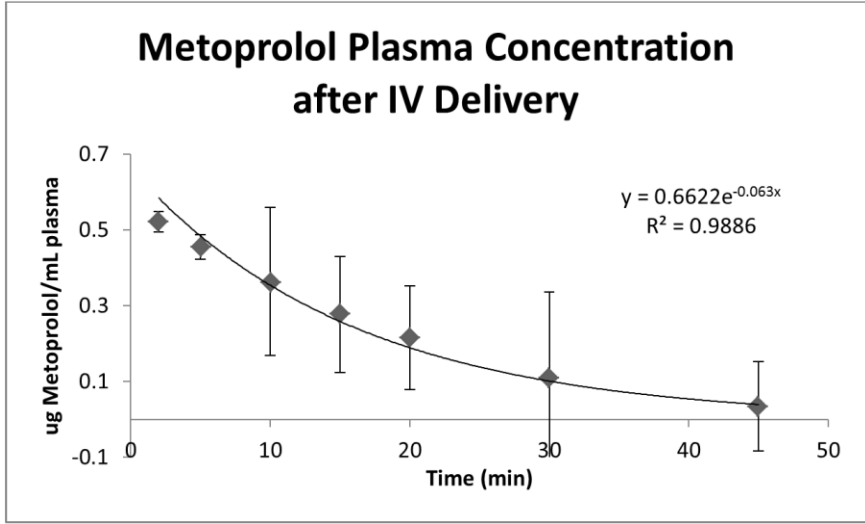
Figure 3.9: Metoprolol concentrations within the plasma after intravenous (IV) therapy; half-life was determined to be 11.1 minutes (n = 2).

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Discussion

We observed that the intrapericardial delivery of metoprolol lowers relative heart rate during induced sinus tachycardia for a sustained period of time, without affecting ventricular contractility or mean arterial pressure. Additionally, we noted that IP delivery resulted in slightly longer times to lower heart rates, compared to IV bolus administration (Figure 3.2). This was considered the result of time it takes for metoprolol to diffuse from the pericardial space into the myocardium. During AF, the ventricular rate for each group did not change with respect to baseline. The IV group remained the lowest throughout the testing period, likely due to the metoprolol reaching the AV node in this group.

With regards to potential effects of metoprolol on altering cardiac refractory periods, we did not observe significant responses, a finding that we consider is associated with small sample sizes and/or high measured variability within our data. Nevertheless, the prevention of changes in either inter-atrial conduction times or atrial refractoriness may or may not be clinically significant. It was also observed here that activation times did not change across the myocardial wall, which may suggest that the transmural concentrations gradient will not induce ventricular arrhythmias. However, we suggest that more complete mapping studies should be pursued. It appears, therefore, that IP metoprolol might best be suited for the treatment of sinus tachycardia and not necessarily for atrial fibrillation (at least based on the findings of this study). Note that the AV node is a deep cardiac structure relative to the more superficial sinoatrial node so, if desired, IV therapeutic delivery may accomplish better rate control than IP administration.

Intrapericardial (or target) delivery to affect heart function could be easily accomplished utilizing a small catheter placed within the chest cavity, e.g., after cardiac surgery. This approach would add minimal time and/or cost to the procedure. In addition, excess drug (particularly if the drug is constantly infused) could be drained via placed chest tubes. In other words, such a therapeutic delivery would be similar to mediastinal irrigation of antibiotics, a procedure often performed for mediastinitis.

Several important potential limitations of these investigations should be considered. First, in our study, the metoprolol delivered intrapericardially was an IV formulation; in the future, perhaps such drug formulations could be better optimized for IP delivery, e.g., accounting for characteristics such as pH. Also, the bolus dosages used in this study were the same as standard clinical IV doses, therefore, additional dose-response studies are needed to optimize the dosage. Nevertheless, as previous studies have shown [5, 6], the IV concentrations of a given drug may be more critical to control than those of the same agent administered within the pericardial fluid. Further, while the IP delivery of metoprolol may lower heart rate, it is clinically important to address the underlying cause of tachycardia as well. Finally, as we noted using the current model, the pericardial delivery of metoprolol in a post-operative setting may not prevent induced AF.

In conclusion, we propose that the pericardial delivery of anti-arrhythmic agents such as metoprolol holds promise for its potential to enhance the efficacy of the drug (i.e., the administration of higher concentrations to the target organ) while mitigating unwanted side-effects. Additionally, IP delivery of such an agent should enable concentrations at

the target site to be more precisely controlled; in this case, metoprolol following IP

delivery was not present in detectable concentrations within the plasma.

Chapter 4: Omega-3 polyunsaturated fatty acids: review of the cardiac and electrophysiologic effects of fatty acid administration

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Preface

This chapter provides a review of the cardiac and electrophysiologic effects of omega-3 polyunsaturated fatty acids. It summarized the current state of research and includes background on the mechanisms of action. The knowledge and understanding gained through this review formed the foundation that the subsequent experiments were based on.

Introduction

As far back as the 1950s, the interplay of saturated and unsaturated fats in diet was correlated to the presentation of ischemic heart disease [1]. Further in the 1970s, Bang and Dyerberg widely publicized their findings on the effects of marine omega-3 poly-unsaturated fatty acids (ω3PUFAs) on positive cardiac health, showing the dramatic differences in cardiovascular health of Inuit Eskimos compared to a Danish cohort. Specifically they observed a lower incidence of death due to acute myocardial infarction among the Eskimos, and this remarkable difference was attributed primarily to the Eskimos' increased intake of ω3PUFAs [2], [3].

Yet, as is the case with observational epidemiologic data, these studies showed a correlation, not causation. However, in the four decades following Bang's publications, a tremendous amount of research has been conducted on the underlying physiologic effects of ω3PUFAs. In addition to reportedly being protective against cardiovascular disease and death [4–8], additional health benefits of high levels of ω3PUFAs have been observed, including: (1) increased clotting time and decreased thrombus formation [9], [10], (2) reduced inflammation [11], [12], (3) the inhibition of apoptosis signaling during ischemia [13], (4) reductions in blood pressure [14], (5) reduced telomeric aging [15], and (6) antiarrhythmic effects [16]. Importantly, these latter antiarrhythmic effects were widely publicized after the GISSI Prevenzione trial. This prospective, randomized controlled trial showed a 45% reduction in cardiac sudden death in the therapy group compared to controls [5]. However, not all follow up studies have affirmed these positive results [5], [17–21].

Background

Long chain fatty acids consist of long (>12 carbons) hydrocarbon chains with a single terminal carboxyl group. Fatty acids are typically described as saturated, mono-unsaturated, or poly-unsaturated, depending on the number—if any—of double bonds present in the hydrocarbon chain. The major unsaturated fatty acids fall into omega-6 (ω6) or omega-3 (ω3) groups, where the -3 and -6 suffixes refer to the position of the double bond in relation to the non-carboxyl end (Figure 4.1).

Omega-6 Polyunsaturated Fatty Acids   **Omega-3 Polyunsaturated Fatty Acids**

Linoleic Acid (LA)   α-Linolenic Acid (ALA)

Arachidonic Acid (AA)   Eicosapentaenoic acid (EPA)

Docosapentaenoic Acid (DPA)   Docosahexaenoic acid (DHA)
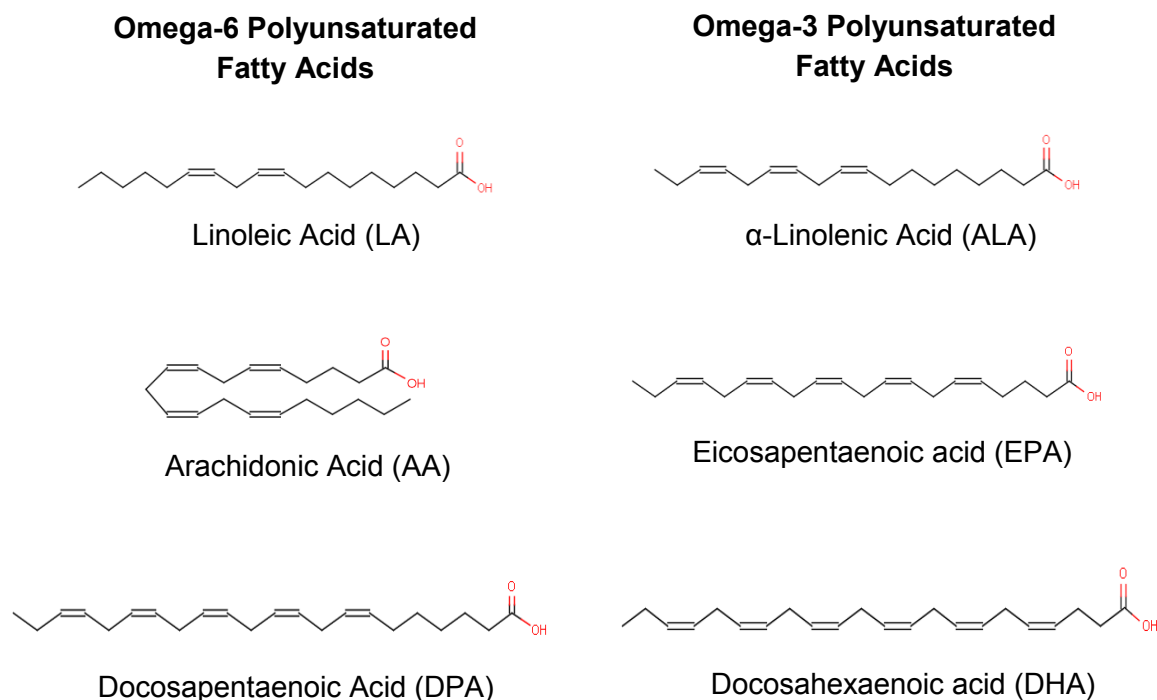
Figure 4.1: diagrams of the most common ω6 and ω3 polyunsaturated fatty acids.

Importantly, in 1930, Burr and Burr showed that mammals do not possess the enzymes required to synthesize the double bonds at the ω3and ω6 positions on the fatty acid chain, and therefore must obtain these essential fatty acids through their diet [22]. At 18

carbons long, linoleic acid (LA) is the parent ω6 fatty acid (C18:2n-6) and is present

primarily in soy, corn, safflower and sunflower oils. Through a series of enzymatic steps,

LA is elongated and desaturated into arachidonic acid (C20:4n-6; AA).

In the cholorplasts of plants, LA can be desaturated to the ω3 α-linolenic acid (C18:3n-3,

ALA). Similar to LA, ALA can be elongated and desaturated into the 20-carbon version,

in this case known as eicosapentaenoic acid (C20:5n-3; EPA), which can be elongated

into docohexanoeic acid (C22:6n-3; DHA).

The 20-carbon PUFAs AA and EPA are the main sources of cell messengers,

prostaglandins, leukotrienes, and lipoxines after conversion by the enzymatic actions of

cyclooxygenase, lipoxygenase and epoxygenase. Additionally, it has been established

that eicosanoids from the ω6 AA have pro-inflammatory functions, whereas the

eicosanoids derived from the ω3 EPA are much less inflammatory [23]. It should be

noted that both AA and EPA compete for the same cyclooxygenase, epoxygenase, and

lipoxygenase enzymes to form their respective classes of eicosanoids; influenced by

their relative concentrations. A schematic diagram of the fatty acid types and

conversions is included in Figure 4.2

Eicosanoids

pg = prostaglandin   tx = thromboxane
pgi = prostacyclin   lt = leukotriene
= less inflammatory
= more inflammatory

Omega-3 family

α-linolenic acid
18:3 ω-3

stearidonic acid
18:4 ω-3

eicosatetraenoic acid
20:4 ω-3

eicosapentaenoic acid
EPA   20:5 ω-3

docosapentaenoic acid
DPA   22:5 ω-3

docosahexaenoic acid
DHA   22:6 ω-3

Sprecher's Shunt

pgd₃ pge₃ pgf₃α
pgi₃ txa₃
lta₅ ltb₅ ltc₅ ltd₅

A/J-Ring Neuroprostane
17S Resolvins
blocks prostanoids

Omega-6 family

linoleic acid
18:2 ω-6

γ-linolenic acid
GLA   18:3 ω-6

dihomo γ-linolenic acid
DGLA   20:3 ω-6

pge₁ pgf₁α
txa₁
blocks lt₄

pgd₂ pge₂ pgf₂α
pgi₂ txa₂ lta₄ ltb₄
ltc₄ ltd₄ lte₄

arachidonic acid
AA   20:4 ω-6

docosatetraenoic acid
22:4 ω-6

docosapentaenoic acid
22:5 ω-6

Δ6 desaturase

elongase

Δ5 desaturase

elongase

Δ4 desaturase

main

Figure 4.2: chart of polyunsaturated fatty acids and their reactions from the parent molecules ALA and LA. The prostaglandins and leukotrienes are produced by EPA and AA in competition for the sam cyclo-oxygenase and lipoxygenase enzymes. Figure by David R. Throop.

Because of man's inability to interconvert ω-6 and ω-3 fatty acids, plants, plankton and algae are the original dietary source of ω3PUFAs. Yet, while soybean, flax seed canola oils contain some ω3PUFAs, mainly ALA, most ω3PUFAs in the human diet, especially EPA and DHA, are consumed by eating fish and other marine animals. And even among fish species, the values of ω3PUFAs per 3oz serving can range from 0.13g in pacific cod to 1.83g in Atlantic salmon [24]. Interestingly, in the typical Western diet, fatty acids categorized as n-6 are consumed 20-25-fold more than n-3 fats [12].

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Electrophysiology

Not only have ω3PUFAs been observed to lower the incidence of CHD, but as noted above evidence suggests they have a direct antiarrhythmic effect. More specifically, a series of animal studies performed by Billman *et al.* demonstrated the antiarrhythmic effects of ω3PUFAs when administered during ischemia [25–27]. To do so, an inflatable cuff was surgically implanted around the left circumflex artery in a dog model of ventricular fibrillation (VF), and after a healing period, VF could be reproducibly induced by inflating the cuff during exercise. By administering ω3PUFAs immediately prior to cuff inflation, the heart was protected in 10 out of 13 dogs using fish oil concentrate, either as purified EPA, DHA or ALA, without significant effects on ventricular contractile functions [28].

In a preliminary human study, electrophysiological testing was performed on patients with implanted defibrillators before and after an infusion of ω3PUFAs (Omegaven, Fresenius, Kabi, Germany). In the seven patients in whom ventricular tachycardia (VT) could be induced, the subsequently infused ω3PUFAs prevented stimulated ventricular tachycardia in five: but it should be noted that the VT induced in the two patients was only after a more aggressive pacing protocol [29]. Given that this was only small pilot study, it preliminarily shows the potential for the antiarrhythmic effects of ω3PUFAs in humans as well.

Multiple human trials have been performed to assess the efficacy of ω3PUFAs in preventing the occurrence of AF after coronary artery bypass graft surgery (CABG). To date, CABG procedures has become common, with 158,000 procedures performed in

the US alone in 2010 [30]. It is important to note that post-operative AF is a common morbidity associated with thoracic surgery: e.g., in CABG patients there has been reported prevalence between 25-40% [31]. The study by Calo *et al.* observed that in patients administered 1.7g/day ω3PUFAs (1:2 EPA/DHA) for at least 5 days before CABG surgery had significantly lower incidences of postoperative AF (OR 0.35; 95% CI 0.16 to 0.76) and shorter hospital stays [18]. In a similar study, in which they provided 4.6 g/day of ω3PUFAs for at least 3 weeks before either CABG and/or valve repair surgeries also corroborated the reduction in hospital stay, but they observed a non-significant reduction in postoperative AF (OR 0.63, 95% CI 0.56 to 1.11) [32].

In order to understand the biochemical and biophysical mechanisms of the antiarrhythmic action of ω3PUFAs, Kang *et al.* used cultured neonatal rat cardiomyocytes in an in vitro model of arrhythmogenesis [33–35]. Perfusion of these cultured cells with known arrhythmogenic agents i.e. isoproterenol, cAMP and cholera toxin, would increase intrinsic beating rates, induced contractures and/or cause fibrillations. Noteworthy, the addition of polyunsaturated fatty acids (but not monounsaturated or saturated fatty acids) prevented all fibrillation. Further, if arrhythmia was produced by one of the agents, the addition of EPA or DHA to the perfusate stopped the arrhythmia. In both scenarios, the fatty acids could be removed from the culture by the addition of fatty acid-free bovine serum albumen and the arrhythmias would initiate (or return) [33–35]. The ability to wash out the ω3PUFAs with delipidated serum suggests that the ω3PUFAs acted directly on these heart cells without the formation of covalent bonds.

Subsequently, Kang and colleagues went on to show the effects of free ω3PUFAs on membrane excitability. They observed that the ω3PUFAs, but not the monounsaturated or saturated fatty acids, reduced membrane excitability by significantly increasing the strength of depolarizing current required to initiate an action potential, as well as prolonging the refectory periods following an action potential. These changes were associated with an increase in the threshold required to initiate an action potential and a lower voltage resting membrane potential [36].

Cardiac ion channel effects

Because the basis of action potential initiation and propagation involves several membrane ion channels, most drugs that have anti- or pro-arrhythmic effects act as ion channel agonists or antagonists. Therefore, numerous single cell electrophysiology experiments have been performed to elucidate the mechanism of action of ω3PUFAs including patch clamp. Interestingly, the administration of ω3PUFAs has been shown to have effects on Na^+, Ca^+, K^+ ion channels as well as pumps such as the Na^+/Ca^+ exchanger.
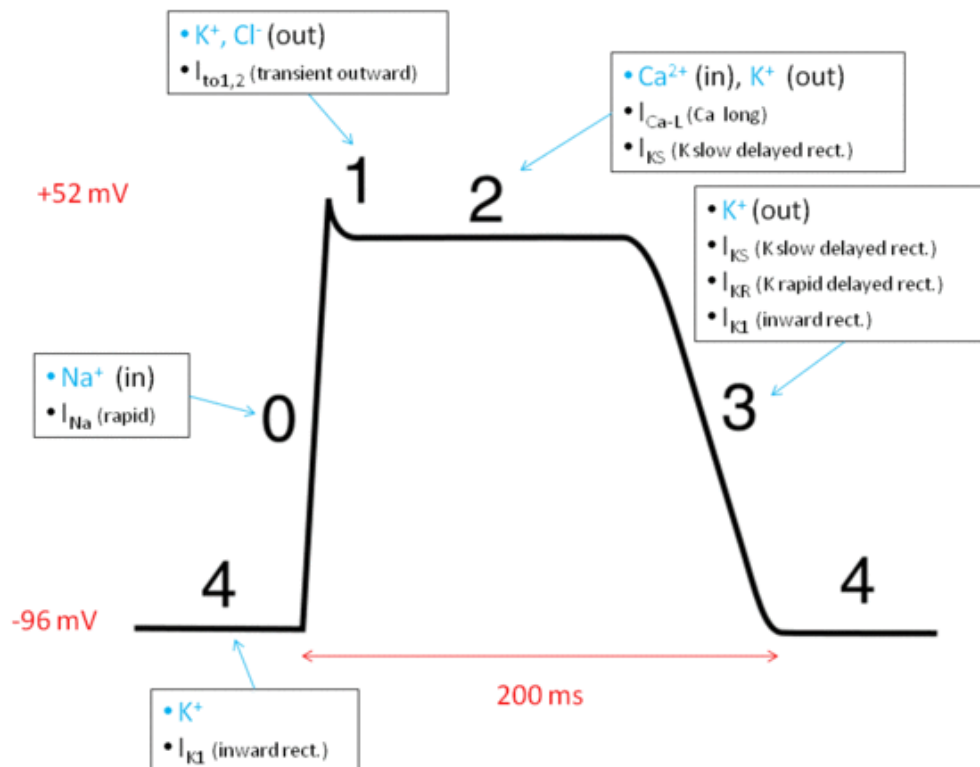
Figure 4.3: summary of ion currents in cardiac action potential

The labels in the figure:

- K⁺, Cl⁻ (out)
- $I_{to1,2}$ (transient outward)

- Ca²⁺ (in), K⁺ (out)
- I_{Ca-L} (Ca long)
- I_{KS} (K slow delayed rect.)

- K⁺ (out)
- I_{KS} (K slow delayed rect.)
- I_{KR} (K rapid delayed rect.)
- I_{K1} (inward rect.)

- Na⁺ (in)
- I_{Na} (rapid)

- K⁺
- I_{K1} (inward rect.)

+52 mV

-96 mV

200 ms

Na⁺ channels

The voltage gated Na⁺ current (I_{Na}) is responsible for the primary depolarization (phase 0; see Figure 4.3) of cardiomyocytes and also is known to play an important role in arrhythmogenesis. It is the activation of these channels that leads to a quick inflow of sodium ions that initiates the action potential. In cultured neonatal rat ventricular myocytes, whole cell patch clamp has revealed that extracellular application of EPA or DHA suppresses the I_{Na}. However, monounsaturated or saturated fatty acids did not have significant inhibitory effects [37]. The ω3PUFA EPA also has been shown to significantly shift the steady-state inactivation curves of these channels to the

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hyperpolarizing direction [37]. This shift in the steady-state inactivation indicates that the membrane potential in cells treated with ω3PUFAs must be more negative in order to produce the same I_{Na} seen in control cells.

Further experimentation on rat cardiomyocytes has also shown that cells treated with EPA or DHA required larger depolarization currents to elicit action potentials (increased thresholds for action potential elicitation) [36]. Specifically, the addition of ω3PUFAs to such cultured cells shifted the steady-state inactivation curve of the Na^+ current more negative (Figure 4.4). Furthermore, the addition of these ω3PUFAs to cells in culture also reduced the membrane resting potentials [36]. These effects of ω3PUFAs on these Na^+ currents are considered to play significant roles in their antiarrhythmic effects.
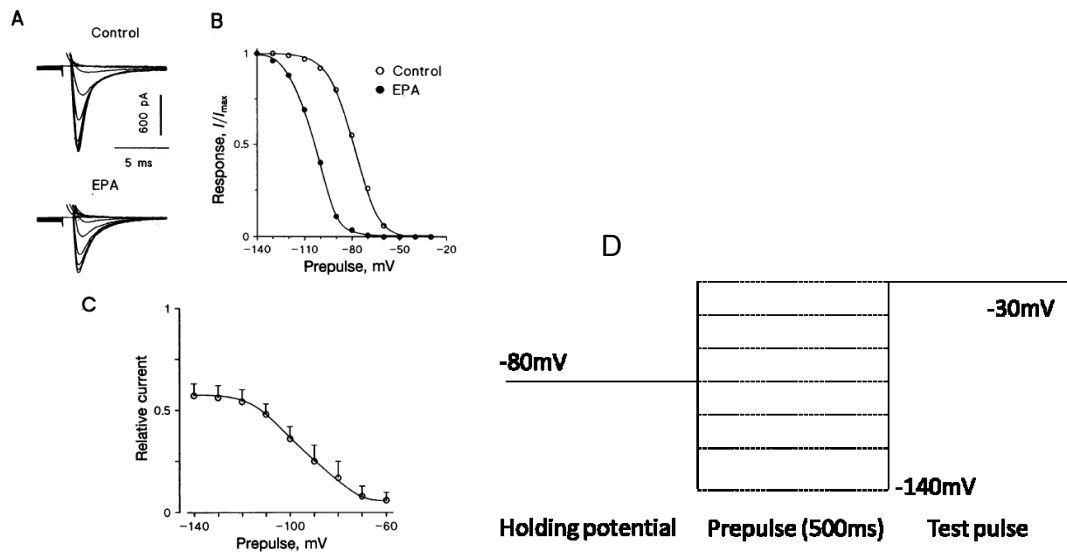
Figure 4.4: The addition of ω3PUFAs shifts the steady-state inactivation curve to a more negative position, reducing response current at a given voltage. (A) Superimposed currents in the absence and presence of EPA (10μM). Currents were elicited with a double pulse: 30 ms testing pulse to -30mV following a 500 ms prepulse varying from -140 to -30 mV from a holding potential of 80mV. (B) Steady-state inactivation peak of sodium currents as a function of prepulse voltage, comparing control to EPA treated. (C) Prepulse suppression effects of 10μM EPA on cardiac sodium currents. Relative currents as a function of prepulse voltage are plotted, and relative currents were calculated as a ratio of treated to control currents. (D) Schematic of pulse sequence. Figure adapted from [37].

It should be clarified that cardiac Na^+ channels are made up of multiple parts: a larger α-subunit that alone creates a functional membrane channel and smaller β_1- and β_2-subunits [38]. It was reported that in a human kidney cell transfected with only the α subunit of the human Na^+ channel, fatty acids reduced $I_{Na,\alpha}$ and shifted the steady state inactivation to the hyperpolarizing direction. In these α subunit-only channels, this observed fatty acid effect was not limited to ω3PUFAs, but monounsaturated and saturated fat additions resulted in similar $I_{Na,\alpha}$ reductions [39].

It is generally considered that β_1-subunit modulates the voltage dependent Na^+ channel [40]. Note that the currents of human Na^+ channels with both the α and β_1 subunits were inhibited by PUFAs, but not saturated or monounsaturated fatty acids [41].

Following these observations, Xiao *et al*. went on to show that that a single point mutation on the α-subunit of human cardiac Na^+ ion channel decreased the inhibitory effect of PUFAs on the Na^+ current. This suggests that the human cardiac Na^+ channels haves a specific binding site for ω3PUFAs [42], [43].

These effects on the Na^+ channel induced by ω3PUFAs may also account for the reduced arrhythmias during ischemia: i.e., within those border regions of cells that are mildly ischemic and thus lack of ATP. In such cases, this results in reduced function of the Na^+/K^+ pumps and the resting membrane potentials slowly rises. Consequently, an action potential can be triggered with very little additional stimulus, thus increasing the risk that such cells will depolarize during a vulnerable point in the cardiac cycle. However, cells with high ω3PUFAs that have a depressed resting membrane potential and require a larger depolarization current will have a stabilizing effect in the border region.

Ca^{2+} Channels

High intracellular concentrations of calcium within myocytes must be achieved for sufficient receptor binding to the regulatory protein troponin, which in turn allows for actin-myosin cross-bridge formation. Initially, the intracellular Ca^{2+} initially comes from outside the cell through the L-type Ca^{2+} channels, but not in sufficient quantities for full

activation: this transmembrane Ca^{2+} intake through the L-type Ca^{2+} channels triggers the ryanodine receptors to release larger amounts of Ca^{2+} from the internal stores within the sarcoplasmic reticulum. As such, an overload of intracellular Ca^{2+} may in turn cause arrhythmias.

Interestingly, cellular reductions in Ca^{2+} L-type gating and releases from the sarcoplasmic reticulum were observed with the addition of ω3PUFAs, but not saturated or monounsaturated fatty acids [44]. Note, while the reductions in the sarcoplasmic reticulum Ca^{2+} current could also be from the reduction in the signaling L-type current, more specifically it was demonstrated that ω3PUFAs reduce the availability of Ca^{2+} for uptake and they inhibit the release mechanism [45].

K^+ Channels

The three main K^+ currents in the cardiac membrane are: 1) the transient outward K^+ current, 2) the delayed rectifier K^+ current and 3) the inwardly rectifying K^+ current. It was reported that both the transient outward K^+ currents and delayed rectifier K^+ currents were inhibited within isolated ferret cardiomyocytes by both DHA or EPA,: whereas the inwardly rectifying K^+ current was unaffected [46]. These differences in effects among the K^+ channels may in part result from their differences in their innate channel structures [47]. Importantly, other saturated or monounsaturated fatty acids did not elicit any significant effects on the major K^+ currents. In theory this inhibition the outward K^+ currents would prolong the action potential duration, therefore the inhibitory effects on the Na^+ and L-type Ca^{2+} currents would have to override the K^+ current effects in order to

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explain the observed reduction in action potential durations observed in cultured cardiomyocytes [36].

ω3PUFAs and Atrial Fibrillation

While the beneficial effects of ω3PUFAs on sudden cardiac death have considerable support to date, the evidence supporting reductions in AF are less robust. Nevertheless, the potent effects of ω3PUFAs on cardiomyocytes ion channels, would suggest that the antiarrhythmic properties may extend to altering AF.

Clinical uses of ω3PUFAs to treat and prevent AF have been reported as mostly—but not always—positive. For example, concerning the occurrences of AF after coronary artery bypass grafting (CABG), Calo and colleagues [18] reported patients who started taking 2g/day supplemental ω3PUFAs for at least 5 days before elective coronary bypass grafting had lower incidences of post-operative AF. A similar study was performed in Germany in which patients were given 100 mg fish oil per kg of body weight per day or a similar amount of soya oil as a control group, all at least 12 hours before CABG procedures until discharge. Post-operative AF occurred significantly less in the ω3PUFA treated group, which also had a shorter ICU stay compared to controls [48].

Yet as noted above, not all reported outcomes post-CABG have been favorable. Specifically, in a study by Saravanan et al., patients were treated with 2g/d ω3PUFA or olive oil for at least 5 days before CABG until discharge: they observed a slight but non-significant increase in the amount in AF in those in the ω3PUFA arm of the study [17].

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In another study, performed on patients undergoing CABG and/or valve surgery, they compared outcomes of patients taking 4.6 g/day ω3PUFAs or placebo starting 3 weeks before surgery. Though the treated patients had a significantly shorter ICU stays, there were non-significant reductions in AF incidences [32].

Mixed results have also been observed in studies specifically aimed at measuring the potential prevention of AF by dietary ω3PUFAs. Interestingly, in one prospective study, Mozaffarian et al. [49] found reduced incidences of AF in elderly individuals who regularly ate bake or broiled fish during 12 years of follow up, but not those who ate fried fish.

Additionally, the clinical use of ω3PUFA administrations for the treatment of patients with AF has also been explored. For example, the relative safety and effectiveness of the prescription ω3PUFA supplement, Lovaza (GlaxoSmithKline, North Carolina), was tested in patients with AF. Specifically, during the six month follow up period, this high dose treatment (4g/day) for AF was observed to have no significant improvements in times to first AF events or in recurrences of symptomatic AF [50]. However, this type of ω3PUFA therapy was beneficial compared to placebo in reducing AF when either was used in combination with amiodarone and a rennin-agniotensin-aldosterone system inhibitor. Furthermore, in a population who underwent cardioversion to return them to sinus rhythm, patients provided with additional ω3PUFA therapy were observed to be more effective at maintaining sinus rhythm during a one year follow up period [19].

Anti-inflammatory effects of PUFAs

Eicosanoids are considered to be among the mediators of inflammation and are

generated mainly from 20-carbon PUFAs. The two main 20 carbon PUFA sources are

AA and EPA. The eicosanoids converted from AA generally promote inflammation,

while those eicosanoids from EPA are typically less inflammatory or inactive.

Inflammatory cells, like many others, often contain a large percentage of the ω6PUFAs

relative to their ω3PUFA counterparts; thus, AA is typically the major substrate for

eicosanoid synthesis. When the concentration of ω3PUFAs is increased within tissues,

there are less ω6PUFAs and fewer of the pro-inflammatory ω6 eicosanoids can be

produced.

More specifically, both ω3- and ω6PUFAs compete for the same cyclooxygenase and

lipoxygenase enzymes. Therefore, an increase of ω3PUFAs makes those enzymes less

available and this competition further decreases ω6 eicosanoid production. Additionally,

it is considered that several of the ω3 eicosanoids produced will also counteract the ω6

eicosanoids, increasing the anti-inflammatory effects of ω3PUFAs.

Finally, in addition to these competitive effects with AA that can reduce inflammation,

ω3PUFAs are considered to elicit several anti-inflammatory effects. For example,

dietary fish oil has been shown to result in: 1) decreased productions of reaction oxygen

species, 2) decreased productions of pro-inflammatory cytokines, leukocyte chemotaxis,

and 3) lower adhesion molecule expressions [11] (Table 4.1)

Antiinflammatory effect	Mechanism
Decreased generation of AA-derived eicosanoids (many with inflammatory actions)	Decreased AA in cell membrane phospholipids; inhibition of AA metabolism, decreased induction of COX-2, 5-LOX and 5-LOX activating protein
Increased generation of EPA-derived eicosanoids (many with less inflammatory actions than AA counterparts)	Increased content of EPA in cell membrane phosphlipids
Increased generation of EPA and DHA-derived resolvins (with antiinflammatory actions)	Increased content of EPA and DHA in cell membrane phospholipids
Decreased generation of inflammatory cytokines	Decreased activation of NFκB; activation of PPARγ, altered activity of other transcription factors, differential effects of AA vs EPA derived eicosanoids
Decreased expression of adhesion molecules	Decreased activation of NFκB, altered activity of other transcription factors
Decreased leukocyte chemotaxis	Unclear; perhaps decreased expression of receptors for some chemoattractants
Decreased generation of reactive oxygen species	Unclear; perhaps altered membrane composition affecting signaling process

Table 4.1: Summary of the anti-inflammatory effects of ω3PUFAs, [11], [51]

Preconditioning with PUFAs

DHA has been shown to exert preconditioning effects in hearts of rats provided with high

levels in their diets. Specifically, it was reported that after 6 weeks of one of three

experimental diets: 1) ω3PUFA; DHA and olive oil; 2) ω6PUFA; sunflower oil and olive

oil, or 3) saturated fats; beef tallow and olive oil. The hearts from rats fed the ω3PUFA

diet had significantly lower heart rates, lower end-diastolic pressures and elicited fewer ischemic and reperfusion arrhythmias, as well as a smaller infarct size compared to the ω6PUFA and saturated fat groups. In other words, it was also considered that preconditioning with ω3PUFAs was as effective as ischemic preconditioning in the ω6PUFA and saturated fat groups [52].

In another series of experiments, McGuinness and colleagues gave rabbits an intravenous dose of an ω3PUFA emulsion (Omegaven, Fresenius Kabi, Bad Homburg, Germany) or saline for 4 days before I/R injury was induced by occluding the large marginal branch of the left coronary artery for 30 minutes and this was then followed by and reperfusion for 3 hours. It was observed that in the group treated with ω3PUFAs saw a 225% increase in heat shock protein before ischemia (heat shock protein is a known preconditioning agent), and was also observed that there was a 40% reduction in infarct area after I/R injury [53]. In an acute swine model of ischemia/reperfusion, Xiao et al. administered DHA to the pericardial space immediately prior to ischemia. This infusion reduced reperfusion injuries and infarct area by 50% compared to controls.

It should be noted that in general, the differences between the terms cardioprotective and pre-conditioning are subtle. By definition, with preconditioning, a physical or chemical stimulus activates natural cellular responses to protect against that cell from subsequent injury. The researcher Hearse defined cardioprotection as "identifying key changes in the progression of ischemic injury and developing anti-ischemic agents to combat these changes, thereby increasing the tolerance of the heart to ischemia" [54]. It is considered that the beneficial effects of ω3PUFAs encompass both preconditioning

and cardioprotective roles. For instance, DHA has been shown to delay Ca^{2+} induced opening of mitochondrial permeability transition pores (MPTP) [55], a contributing factor to reperfusion injury.

Potential mechanisms of action

Several explanations have been put forward for why ω3PUFAs elicit their effects on ion channels, and thus the relative electrophysiologic properties of the whole heart. It was proposed by Jahangiri et al. that the incorporation of ω3PUFAs into the cell membrane changes fluidity and thus membrane channels. To study such, they use fluorescence anisotropy to monitor membrane fluidity, correlating ω3PUFA effects to fluidity [56]. Interestingly, some of the observed effects on ion channels in other experiments noted above occur at much lower concentrations than those necessary to change membrane fluidity [37], [43], [44].

A second hypothesis put forth by Andersen and colleagues suggests that such cell membrane effects are more localized to the immediate vicinity of the channel proteins: they showed that two different detergents produce similar effects as the applications of ω3PUFAs. This was explained as the change in tensions between the ion channels and their incorporating membranes; i.e., when the hydrophobic lengths of the channels do not match up with membrane thicknesses. These mismatched lengths result in slight conformational changes in the ion channels that is relieved by the localized action of incorporated ω3PUFAs [57], [58]. However, the detergents applications had similar effects in several instances where ω3PUFAs did not. In specially designed Na^{+}

channels with a single point mutation on the alpha subunit, importantly the potent effects of ω3PUFAs were lost [42], [43], but the detergent effects were unaltered.

The third hypothesis considers the possibility that the ω3PUFAs may bind directly to the ion channels, exerting a slightly different effect on each. This was further supported by the easy removal of the ω3PUFAs and their action in cell cultures with the addition of delipidated serum albumin. If was subsequently hypothesized that if the ω3PUFAs were incorporated into the membrane, this transition would be slower [43]. Further support of this hypothesis is provided in a study that showed a single point mutation in the α-subunit of the Na^+ channel mad it significantly less sensitive to the inhibitory effects of ω3PUFAs [43].

Negative effects of ω3PUFA

While not all trials using ω3PUFAs have shown beneficial effects, nearly all studies to date have shown no harmful results. As such, many options for ω3PUFA supplementation or fish oil dietary augments are available over the counter, and recent prescription grade ω3PUFAs have been approved in the USA and Europe. Furthermore, the FDA has declared ω3PUFA supplements "generally recognized as safe." Likewise, the American Heart Association recommends two servings per week of fatty fish. The most common negative effects of fish oil supplements include fishy taste and burping, as well as gastrointestinal upset. At very high doses, risk of bleeding can increase, though the majority of studies with increased dietary fish or ω3PUFA supplements show that it is well tolerated.

In summary, there has been much basic and translation interest as to the potential mechanisms and clinical benefits of ω3PUFAs on cardiac function. To date, one may consider that the potential benefits increase level of ω3PUFA within one body greatly outweighs any side-effects. However, more research is required to further substantiate the optimal uses of ω3PUFA for the prevention or treatments of human cardiac disorders.

Chapter 5: Effects of Pericardial treatment with docosahexaenoic acid on electrophysiology and global ischemia

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Preface

Building off the pericardial delivery model created for metoprolol, this chapter investigates the effects of pericardial treatment with omega-3 polyunsaturated fatty acids. Commonly, myocardial omega-3 levels are built up with increased dietary ω3PUFAs, requiring several weeks for the compounds to reach high myocardial concentrations in rodents [1–4]. To observe acute effects, one alternative would be high dose IV injection. A noted complication with injection is the possibility of hemolysis [5], as well as the reduction in available ω3PUFAs as they readily bind to the circulating albumin and become less available. Intrapericardial delivery bathes the heart in the treatment, allowing for higher concentrations, as well as lower concentrations of albumin to bind to the fatty acids. The pretreatment used in chapter 5 was designed based on a similar protocol previously completed in our laboratory that saw a 57% reduction in normalized infarct size and a 50% reduction in reperfusion arrhythmias in a swine model of acute ischemia [6]

Introduction

Localized therapy delivery is seen as a method to accurately deliver treatments to individual tissues in tightly controlled quantities. Because the therapeutic agent only contacts the targeted tissue, potentially harmful systemic interaction with the drug are often reduced or averted with localized delivery methods. Additionally, localized drug delivery greatly enhances the proficiency with which drugs can be delivered. Drug doses can be tailored to the specific tissue and higher doses can more safely be administered because other tissues beyond the target will be minimally affected. The pericardial space is an ideal setting for targeted therapy delivery. The space, created by the pericardium as it surrounds the heart, is a small volume isolated from the rest of the thoracic cavity, with only a small turnover with the systemic circulation [7].

Pericardial delivery has been evaluated in several animal models including rats [8], dogs [9], and pigs [6], [10], with a few case studies described in humans [11]. A number of experiments have been performed with anti-arrhythmic drug delivery to the pericardium, such as digoxin [10], procainamide [10], sotalol [8], atenolol [8], and amiodarone [7]. These studies report high concentration of agent in the pericardial space[4] as well as high concentrations in the myocardium [8], [10] with comparatively low concentrations in the blood plasma, indicating that the agents are truly localized to the pericardial space.

There have also been several agents of cardioprotection described using the pericardial space. This includes pericardial delivery of nitroglycerin [12] and L-arginine [9] to reduce ventricular arrhythmias and even using the pericardial space to circulate chilled fluid to

induce cardioprotective cooling in an ischemia reperfusion model [13], resulting in reduced infarct size.

n-3 Polyunsaturated Fatty Acids for Cardioprotection

High levels of dietary omega-3 polyunsaturated fatty acids (ω3PUFAs) have been suggested to be protective against cardiovascular disease [14–16]. Specifically, ω3PUFAs have been shown to improve postischemic recovery from ischemia/reperfusion injury in rat [1] and swine [6] hearts. Furthermore ω3PUFAs reportedly have antiarrhythmic properties, reducing incidences of atrial fibrillation (AF). Mozaffarian et al. [17] found reduced incidence of AF in elderly who regularly ate bake or broiled fish. Calo and colleagues [18] reported patients who started taking n3PUFA supplements 5 days before elective coronary bypass grafting had lower incidences of AF. These epidemiological studies indicate the efficacy of both short- and long-term ω3PUFA exposures on cardiac functionality.

In most studies, ω3PUFAs are administered intravenously (IV) or through the animal's diet. In rodents, dietary administration of ω3PUFAs has been shown to take several weeks for the compounds to reach high myocardial concentrations [1–4]. Due to the amount of time required, dietary delivery would not be beneficial in an acute setting. In this setting, IV delivery could increase plasma levels, but high doses of ω3PUFAs are required to achieve appropriate myocardial concentrations [5]. An unfortunate consequence of the high dose of ω3PUFAs is that it increases hemolysis, making it an undesirable treatment [19]. Delivery of ω3PUFAs to the pericardial space has been

proposed as a way to build up myocardial concentrations without inducing systemic hemolysis [6].

Localized delivery may be able to play a key role in the administration of protective agents during cardiothoracic procedures. Pericardial delivery during cardiothoracic surgical procedure would be a simple task. This study examines a model for preconditioning the heart with omega-3 fatty acids using Intrapericardial (IP) delivery. It is our hope that this model can be used in a preoperative or pre-transplant setting so as to improve patient outcomes. The ω3PUFA docosahexaenoic (DHA) was chosen for this study since it is the most abundant n3PUFA form found in most tissues, including the myocardium [20].

Measuring AF time and electrophysiology parameters, as well as using Visible Heart[®] methodologies, the specific aims of this study are to investigate the effects of DHA delivery into the swine pericardium on arrhythmias *in situ* and ischemic damage in the reperfused heart. This will be accomplished by assessing: 1) changes in the myocardial refractory periods and conduction velocities, 2) differences in atrial fibrillation susceptibility during treatment, as well as 3) differences in hemodynamic performance and 4) differences in protein effluent during the first hour of reperfusion. We hypothesize that treatments with DHA will decrease AF in the animal as well as improve function in vitro as measured by increased maximum left ventricular pressure (LVP) and decreased minimum LVP.

Methods
Surgical procedure

A total of 33 healthy, castrated male Yorkshire swine, weighing 85±10kg, were used for this study. Anesthesia was induced with telazol and thiopental, animals were then intubated and mechanically ventilated, maintaining a P_{CO2} of 40 ± 2 mmHg, with a 2:1 air to oxygen mixture throughout the duration of the study. A surgical level of anesthesia was maintained with isoflurane (>1.2 MAC). Two Mikro-Tip catheter transducers (Millar Instruments, Houston, TX) were used to monitor ventricular pressures; arterial pressure was monitored with a femoral cannula and a Swanz-Ganz catheter was used to monitor right atrial and pulmonary artery pressures. Endocaridal pacing leads (Medtronic 3830, Medtronic, Minneapolis, MN) were secured into the right ventricular apex and right atrium via fluoroscopy and connected to a GEM III pacemaker/defibrillator (Medtronic, MN). A decapolar Marinr CS catheter (Medtronic, MN) was placed adjacent to the tricuspid valve in order to measure electric potentials along the bundle of His (Figure 5.1).
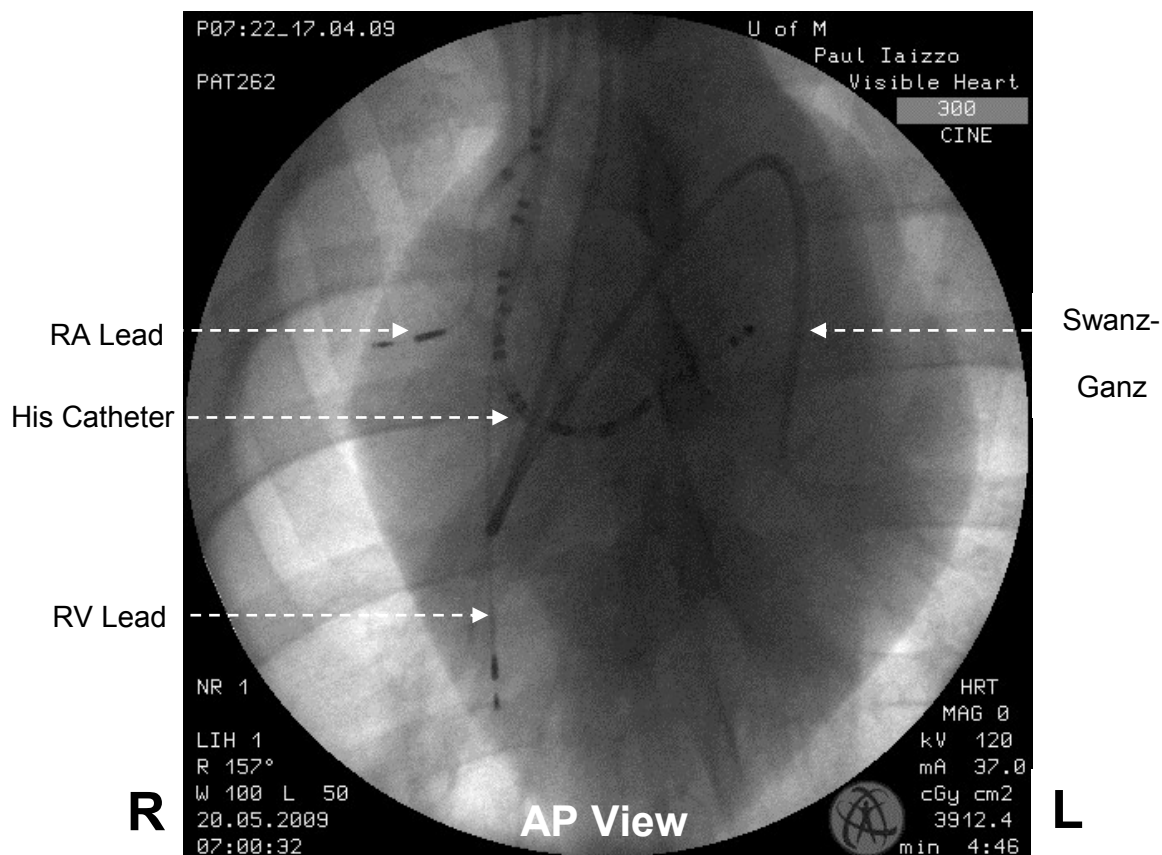
Figure 5.1: Anterior-posterior (AP) fluoroscopic view of an instrumented swine heart with leads in the right atrium (RA) and apex of the right ventricle (RV). A decapolar electrophysiology catheter is positioned through the tricuspid valve and along the septal wall toward the RV outflow tract to detect His potentials. In addition, a Swanz-Ganz catheter, also through traveling through the tricuspid valve and up the pulmonary artery, is measuring pressures in the RA and pulmonary artery.

Access to the heart was then attained via a medial sternotomy and a small incision was made in the anterior portion of the pericardial sack. Four sutures were then used to secure the edges of the incision to the sternum, creating a pericardial cradle (Figure 5.) Additional electrical monitoring was placed: epicardial leads on the left ventricle and left atria, as well as another decapolar electrophysiology catheter placed across the roof of the right atrium (Figure 5.).
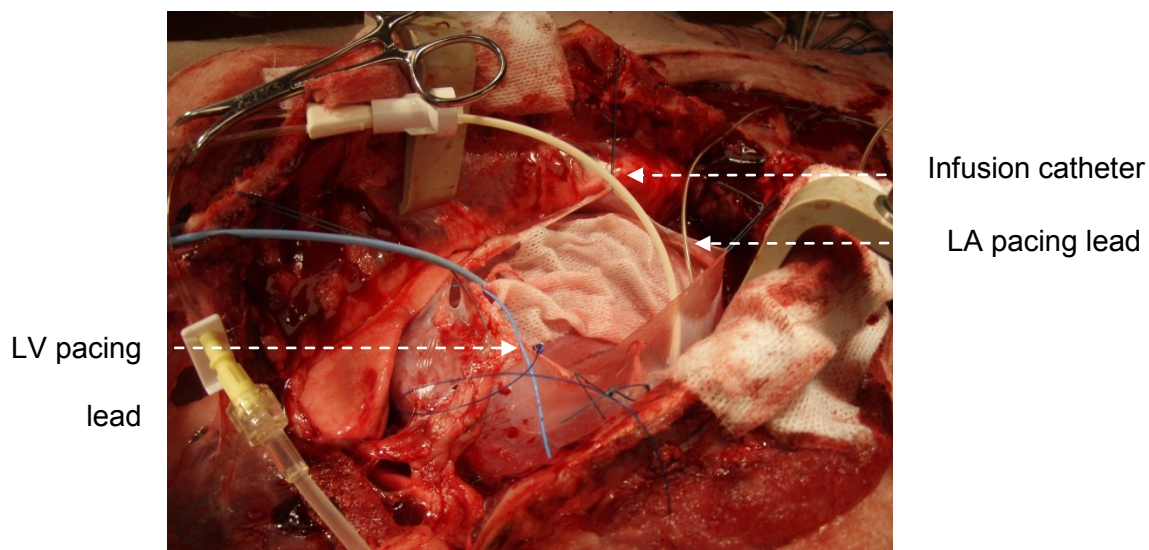
Figure 5.2: the pericardial cradle is created by suturing the pericardium to the medially divided sternum. LV =left ventricle, la= left atrium.

Electrophysiologic measurements

The electrical leads were used to measure refractory periods and susceptibility to AF. The signals from the pacemaker programmer (Medtronic 2090, Medtronic, MN), epicardial pacing leads, decapolar catheter, and EKG leads were recorded simultaneously by an ENSITE electrophysiology system (St. Jude Medical, MN).

Atrial effective refractory periods (AERP), ventricular effective refractory periods (VERP), and atrioventricular nodal effective refractory periods (AVNERP) were measured following a standard protocol explained by Ayers et al. [7]. Briefly, a series of eight paced pulses captured the heart at 150 bpm followed by a 9th pule at an increasingly short interval. Susceptibility to atrial fibrillation was measured by delivering 2-second, 50Hz burst pace of 2-msec, 20V pulses to the left atrial appendage using a stimulator (GRASS Technologies, RI). The duration of AF was recorded after each burst. This was repeated

up to ten times, unless the animal entered a sustained episode of AF (an episode lasting one minute or longer). These electrophysiology parameters were taken just prior to drug delivery as well as at 5, 30, 60, and 90 minutes after drug delivery.

At this point, the heart was given one of four treatments in the pericardial space: 1) a 90 minute infusion of DHA (3mg/mL) for a total of 45mg, 2) a twofold higher dose for a total of 90mg DHA (2xDHA), 3) a control infusion of the vehicle (0.15mL ethanol) or 4) an infusion with normal saline. All treatments were given with a loading dose (6mL/2min) followed by a slow infusion at 01.mL/min for the remaining 88 minutes.

At the end of each treatment, the pericardium was removed and an aortic root cannula was sewn in. The inferior vena cava was clamped off to stop venous return. As the superior vena cava and aorta were cross clamped, the heart was arrested with a cold crystaloid cardioplegia. The pulmonary artery was vented to decompress the heart and prevent distension. Topical cooling was applied with ice and the heart was excised and placed in an ice and Krebs- Henseleit (krebs) buffer slurry. The time to flatline was considered as the period of time from the start of flow of the cardioplegia to the cessation of all spontaneous electrical activity.

In vitro performance

After excising the heart, the great vessels were cannulated, and the heart was reperfused with krebs buffer using Visible Heart® methodologies (Figure 5.) [19]. The heart was warmed to physiological temperatures using Langendorff profusion and defibrillated to initiate a sinus rhythm. After defibrillation, the heart was allowed to

stabilize for 2-4 minutes. Once the heart entered a stable rhythm, the heart was

switched into full working mode and data was collected for 30 seconds. This was

considered time zero, and data collection was repeated at 11 additional time points.

After both the 50 and 55 minute data storage periods, 3-4 liters of the Krebs solution was

drained and replaced.
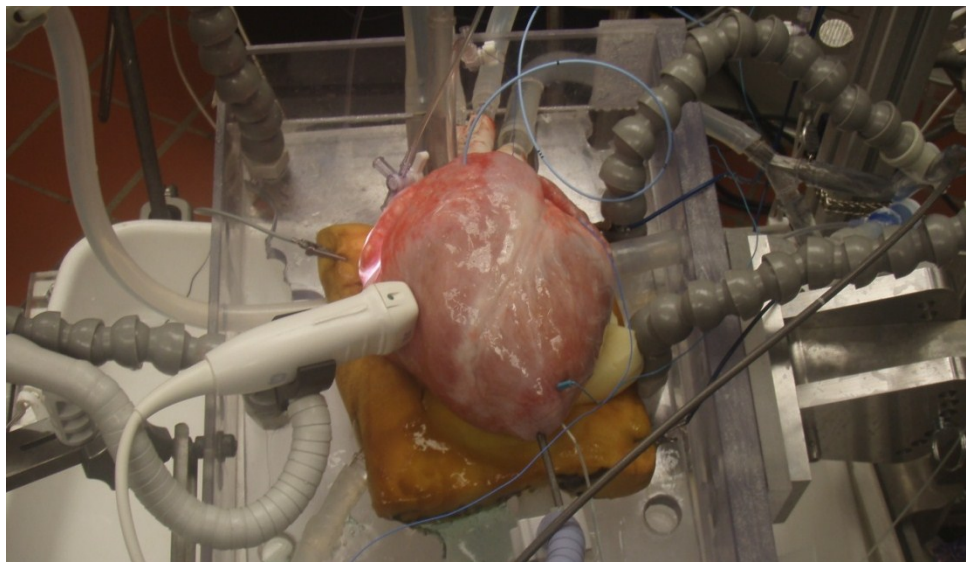
Figure 5.3: Image of porcine heart on Visible Heart perfusion apparatus. Ultrasound transducer position is shown adjacent to the right ventricle.

Ventricular and atrial pressures were monitored using Mikro-Tip catheter transducers

(Millar Instruments, Houston, TX), along with aortic flows using Transonic flow probes

(Transonic Systems, Ithaca, NY). The heart was perfused for 60 minutes, alternating

between right-sided working and full-working modes. During the reperfusion, just prior to

switching to working mode, samples of the Krebs solution were taken for biomarker

analysis. The krebs perfusate was tested for Troponin-I and Myoglobin at the Fairview

hospital clinical labs, and TBARS ELISA assays (Cayman) was also performed. As the

heart was cycled in and out of working mode, short axis ultrasound images were recorded in order to classify myocardial edema (Vivid-i, GE, Fairfield, CT).

Statistics

Data are presented as mean with standard deviation. Significance was determined using Student's t-test or ANOVA, where appropriate. An α of 0.05 was considered significant, with bonferroni corrections when appropriate. Analysis was done using Microsoft Excel 2007 and STATA version 10.1

Results

33 pigs were used in this study, and in vivo data was collected from them all. Four hearts were excluded from in vitro analysis due to complications unrelated to heart heath. (LA input clamped, EMKA failure, and 2 unknown). There was no mortality or major morbidity prior to heart arrest and explant.

There were no significant differences in animal weight or heart weight among groups (Table 5.2). No differences exist among the groups in cold ischemia time, defined as the time between arrest and defibrillation to start reanimation.

Treatment	n	Animal wt (kg)	Heart Wt (g)	cold ischemia time (min)
DHA	8	85.1±14.9	423.9±85.3	78.0±7.0
2xDHA	8	88.4±10.5	440.4±41.7	83.0±12.3
Saline	9	85.1±8.7	418.8±43.9	72.3±5.8
EtOH	8	84.0±11.7	431.1±30.9	76.5±12.6

Table 5.2: baseline characteristics, including animal weight, heart weight and cold ischemia time.

During the explant procedure, time from cross clamp to cessation of electrical and

mechanical activity was recorded as time to flatline. Upon reanimation, the number of

shocks required to restart the heart was also recorded (Table 5.3).

Treatment	n	Time to flatline (sec)	number shocks to restart
DHA	8	44.5±16.3	1.2±0.4
2xDHA	8	54.5±17.4	2.5±2.4
Saline	9	61±44.0	4.3±4.1
EtOH	8	48.3±17.4	2.3±1.5

Table 5.3: Time to flatline upon cardioplegia administration and the number of defibrillation shocks required to restart the heart.

Atrial fibrillation

In this open chest model, pig hearts were induced into atrial fibrillation with a burst pace.

At least one episode of sustained AF was seen in 51.5% of pigs. Ethanol treatment

increased the time in AF over baseline, but not a statistically significant amount (Figure

5.). The DHA and 2x DHA treated hearts saw trends of reduced AF, and statistical

significant reductions from baseline were seen in the 2xDHA dose at 60 and 90 minutes.

However there were no differences when compared to ethanol treatment. There was

almost no fluctuation in the time in AF in the saline treated hearts.
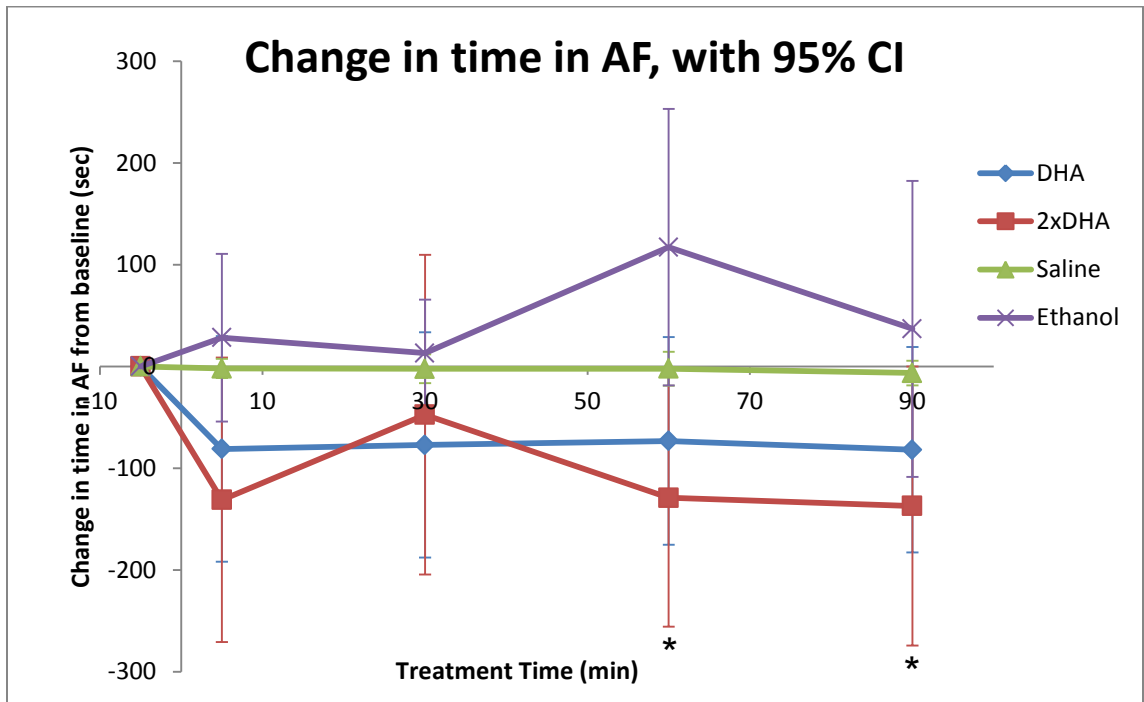
Figure 5.4: Change in time in AF from pretreatment baseline. There was a significant reduction in AF from 2xDHA at the 60 and 90 minute points (*).

Refractory periods

Though most of the treatments made ventricular effective refractory periods (VERP) trend slightly upwards, there were no significant differences among the groups or significant changes from baseline (Figure 5.). Atrial effective refractory periods (AERPs) stayed within 10ms of their starting values, with no statistically significant changes.
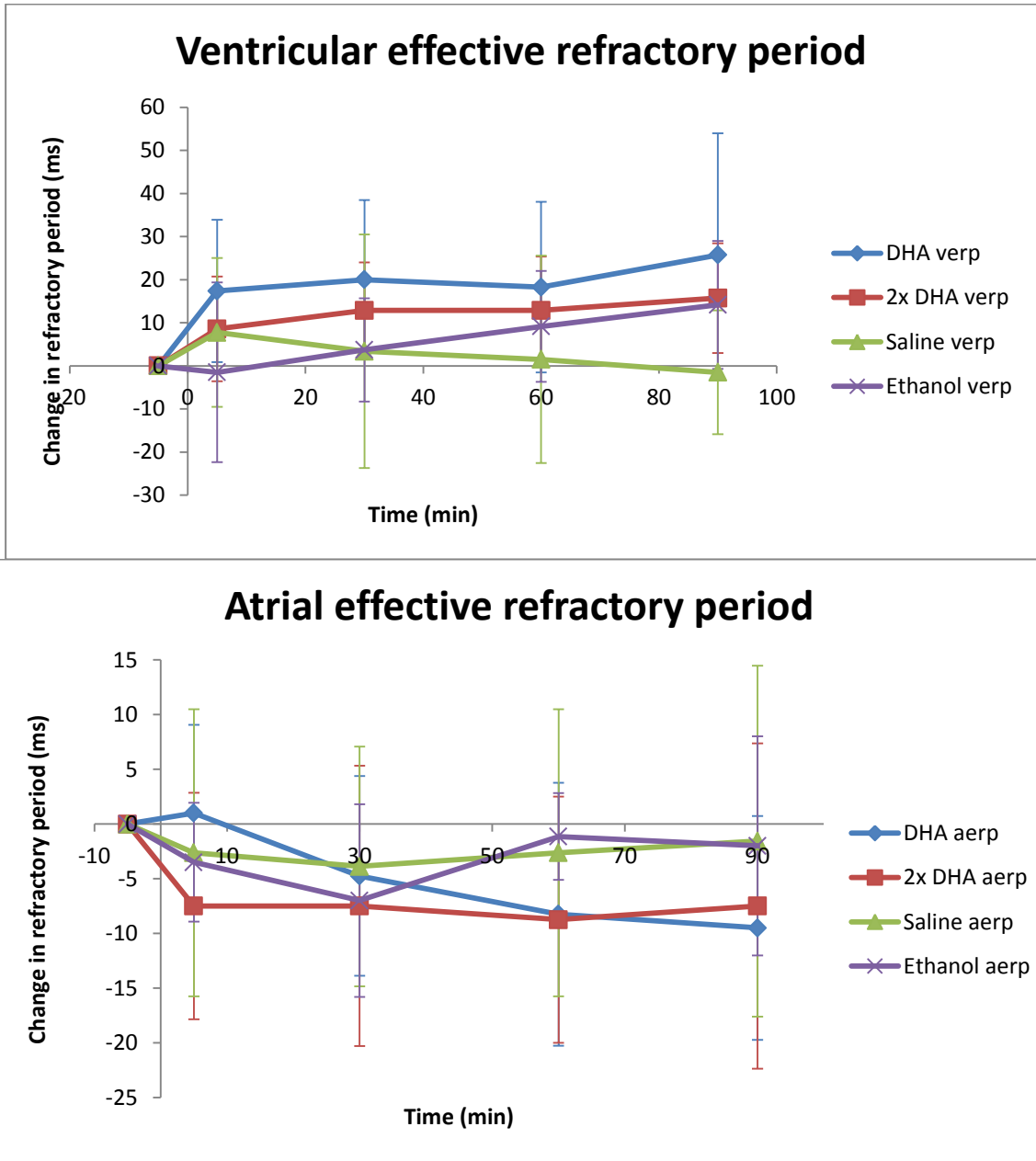
Figure 5.5: ventricular and atrial effective refractory periods.

Hemodynamics

Both the DHA and 2xDHA treatments slightly lowered heart rate in vivo, while the heart

rate for the saline and ethanol treated hearts increased slightly. The DHA treated hearts

had significantly lower heart rate at 60 and 90 minutes and the 2xDHA hearts at 90

minutes (Figure 5.).
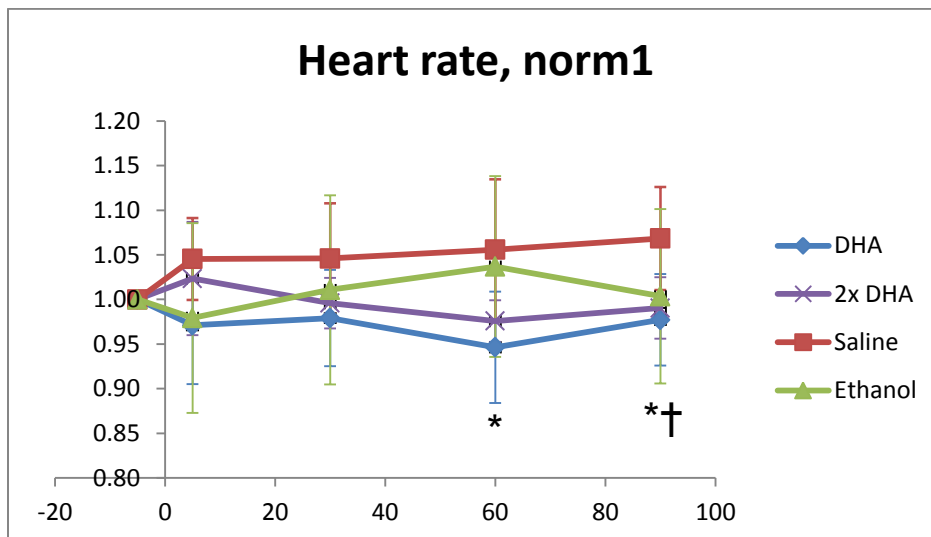
Figure 5.6: heart rate in vivo. Both DHA treatments lowered heart rate, with statistically significant differences when compared to saline. The * indicates significant difference between DHA and saline, † indicates difference between 2xDHA and saline.

Left ventricular pressures

Both during treatment administration and upon reperfusion, the pressures were

recorded. The maximum and minimum LV pressures were measured both *in vivo* and *in*

vitro on the Visible Heart® apparatus.

Maximum LVP did not vary during the in vivo administration; however, there was a 15-

50% increase upon reanimation. The 2xDHA treatment started out higher than most the

other treatments, with statistical significance at 4 and 8 minutes (Figure 5.). From there

the pressure declined and returned to similar values as the other groups.

Hemodynamics

Once the heart was removed and reanimated, hemodynamic data was taken for one hour of reperfusion. The values were normalized to the pretreatment baseline measured in vivo in order to minimize any artifacts from the effect of the treatments.
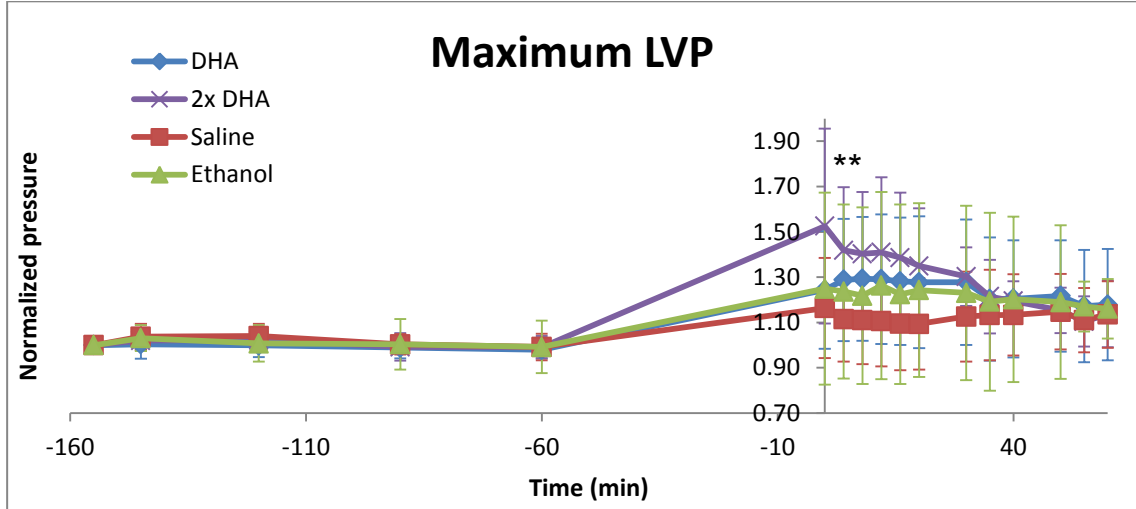
Figure 5.7: maximum left ventricular pressure. The * indicates 2xDHA significantly higher than saline

Minimum diastolic pressure was also calculated. There was no noticeable shift in vivo, with a slight increase of 1-3mmHg when reanimated in the Visible Heart apparatus. During the first hour of reanimation, the minimum pressure continued to increase, with no noticeable difference among the groups (Figure 5.). Maximum and minimum rates of contraction were also measured, with no differences observed.

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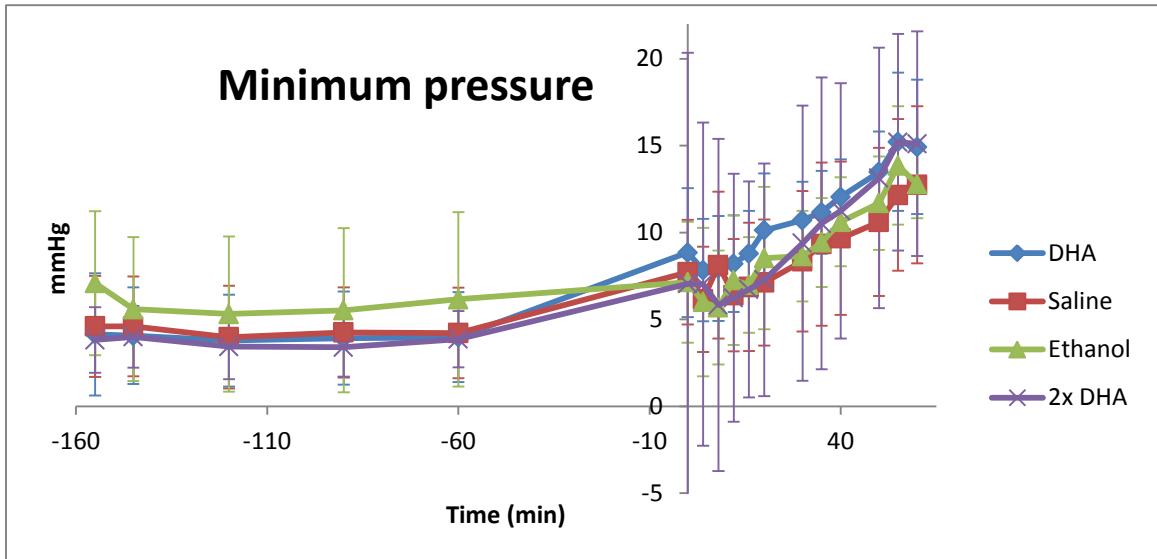
Figure 5.8: Minimum left ventricular pressure. All treatments had some increase in minimum pressures.

Protein levels

The levels of biomarkers measuring cell injury were also collected. There was an observed buildup in levels of lactate, troponin and myoglobin over the course of the first 50 minutes of reperfusion (Figure 5.).
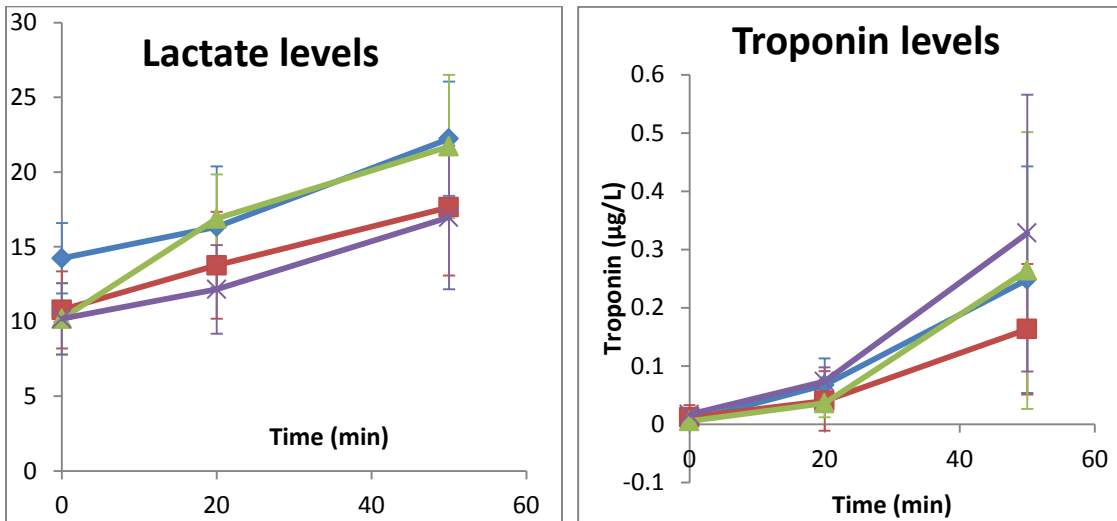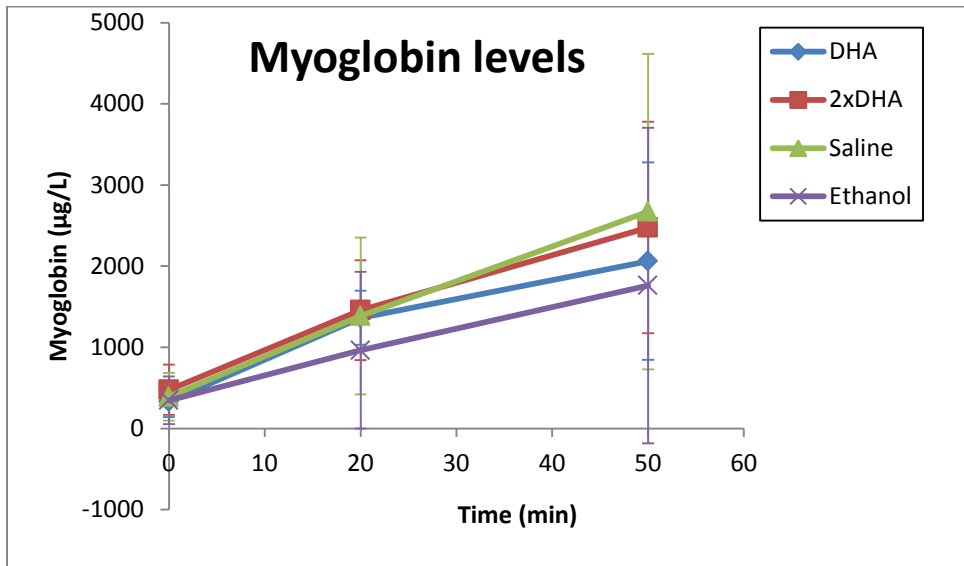
Figure 5.9: biomarkers detected in the circulating krebs buffer. While the levels of each continued to rise, there is little difference among the groups for each biomarker.

Ultrasound

In order to measure the myocardial edema, short axis echocardiographic images were captured during the hour of reperfusion. The size was traced out on the ultrasound unit with the imbedded analysis software (GE, Fairfield, CT). Myocardial cross-sectional area was normalized to the area in right-sided working mode. There were slight increases in area for each treatment, with no significant differences (Figure 5.5).
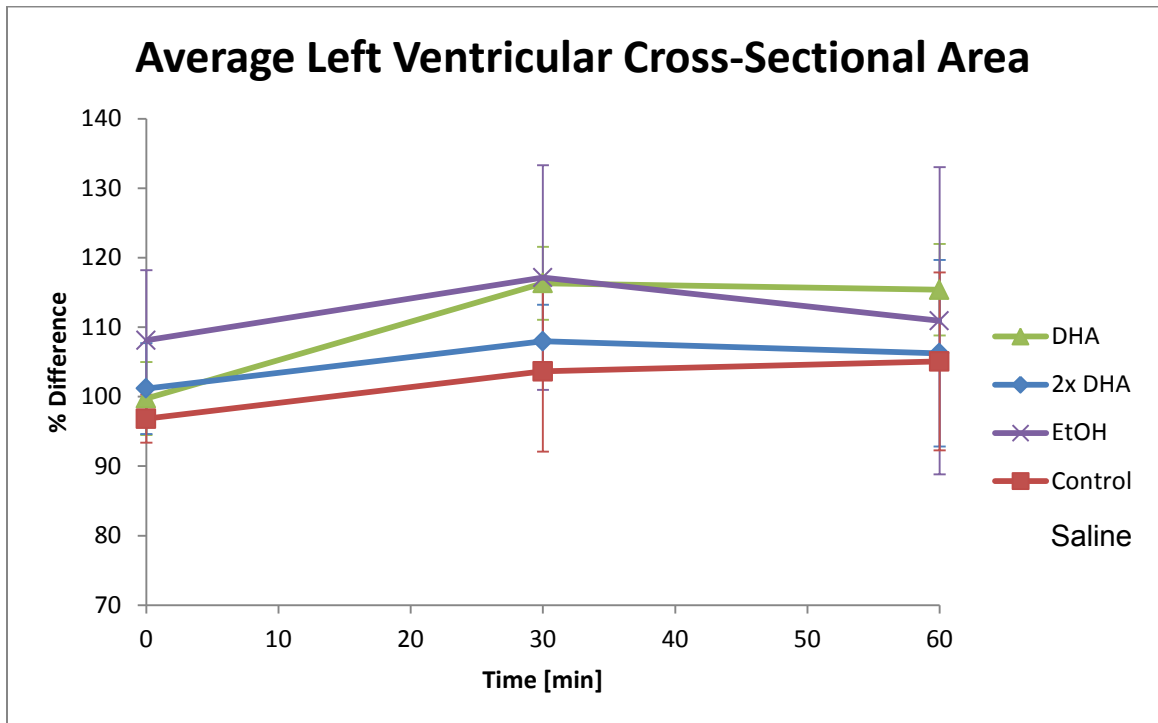
Figure 5.5: Left ventricular cross-sectional area as measured on short axis ultrasound. All treatments increased slightly, with no significant differences.

Discussion

Cardiothoracic surgical procedures carry with them the risk of the patient developing arrhythmias and thromboses. Postoperative AF is a common morbidity, with 25-40% incidence after coronary artery bypass surgery [21]. We have shown in this study is that the administration of a high dose of DHA to the pericardial space can lower AF compared to baseline. Additionally, other studies have shown that ω3PUFAs can reduce heart rate. In this study, we saw a slight decline in heart rate for both dose levels of DHA. The maximum and minimum LVP did not change in vivo after the administration of any of the treatments. This may have significant application in the post-operative intensive care unit, where AF is common and post-op arrhythmias increases ICU stays. Often an arrhythmic or tachycardic patient requires therapy, but using common options

like β-blockers or Na$^+$ channel blockers may also have negative inotropic effects, which are typically undesirable in patients recovering from cardiac surgery. While the antiarrhythmic effects of DHA are mild, it may still have a place as a preventative measure, especially with such an agent where the negative side-effects with other delivery routes are minimal.

Though the exact mechanisms for its antiarrhythmic actions have not been identified, many of the effects of ω3PUFAs are known, leading to several hypotheses. One explanation is the observation that fatty acids have the ability to suppress the voltage-gated sodium channel, shifting the resting membrane potential more negatively [22]. This shift increases the electrical stability of the cells and may reduce the susceptibility to arrhythmias.

Some procedures, including many valve repair and replacement surgeries and all heart transplants also require cardioplegic arrest. Though cardioplegia solutions and topical cooling provide great cardioprotection, there is still room to improve myocardial protection [23]. Upon reanimation, the 2xDHA treated heats generated significantly higher pressures during the first several minutes of operation on the Visible Heart apparatus. The function of these hearts rapidly declined throughout the hour, returning to functioning levels similar to the function of hearts in other treatment groups. This reduction in force may be attributed to the diffusion of DHA back out of the tissue, as the heart was no longer bathed in the solution at this point. In cellular studies, the effects of DHA have been shown to be reversible [24], [25] and DHA has been shown to be able to diffuse quickly [6]. This increase in function may be sustainable with additional

administration of DHA to the heart post reanimation. There was little differences among

the groups in the measured biomarkers. It may be several hours before troponin and

myoglobin are clinically detectable with acute myocardial infarction. Though an increase

in levels was observed, perhaps a larger increase may be observed if the levels were

tracked further out past the ischemic injury.

The improvement in function after global ischemia may have implications for heart

transplant. The cardioplegic arrest and cold storage in these experiments is very similar

to what a transplanted heart will experience. An increase in function on the Visible Heart

apparatus may be predictive of increase in performance of an implanted heart.

Theobserved increases in maximum left ventricular pressure may be a result of the fact

that ω3PUFAs have potent anti-inflammatory effects [26], [27] as well as anti-apoptotic

effects [28], and ω3PUFAs have been shown to help decrease oxidative injury [29].

These effects likely combine to reduce cell damage and death, contributing to the

cardioprotective properties of ω3PUFAs.

Most beneficial effects reported with ω3PUFAs are specific to DHA and EPA

(eicosapentanoic acid). For this study, DHA was chosen because it is the most common

ω3PUFAs in cell membranes, including heart cells [20]. Additionally, previous studies in

our lab have shown pericardial delivery of DHA can reduce arrhythmias and infarct size

in a swine model of ischemia/reperfusion injury.

Many studies investigating the cardiovascular effects of ω3PUFAs make use of long-

term dietary intake. The advantage of such an approach is its non-technical and non-

invasive nature. However, a dietary loading method relies on dietary absorption, blood

transport, and storage in cell membranes where it can be readily available; a process

that may take weeks with high ω3PUFA intake and contains patient to patient variability.

The proposed pericardial delivery simplifies the route to the myocardium, greatly

reducing individual variability as well.

Limitations

Although the Visible Heart® approach provides an excellent model for studying cold

ischemia and heart function, there are several limitations. Because the circulating buffer

has no oxygen carriers, the working heart undergoes minor global ischemia. It is thus

necessary to cycle the heart out of a full working mode into a "resting" right side working

mode. In this mode the right side of the heart is pumping little more than the coronary

effluent. This lack of oxygen may also be responsible for the decline in function seen in

the DHA treated hearts, i.e., the myocardial oxygen demand was higher due to the

higher function, and these higher functioning hearts felt the lack of oxygen much more

than the lower functioning hearts. It is also important to note that a transplanted heart

would have reduced neural connectivity, but it would maintain hormonal responses. The

tissue in this experiment lacks all neurohormonal influences from the body. While this

differs from the *in vivo* conditions, the Visible Heart® setup makes it possible to isolate

cardiac responses from other influences.

With pericardial delivery, one concern is the fact that agents have to diffuse through

tissues instead of being perfused through the vasculature. With high concentrations on

the epicardial surface and very low concentrations on the endocardial surface,

myocardial gradients of the agent could possibly occur. Theoretically, if a treatment

alters conduction velocity, such a drug gradient could produce heterogeneous

conduction within the ventricular myocardium, promoting ventricular arrhythmias. While

such gradients have been seen [7], no arrhythmias have been reported in published

models

Conclusions and future work

We have shown here that the omega-3 polyunsaturated fatty acid DHA, when delivered

to the pericardial space, can reduce induced atrial fibrillation and improve initial in vitro

performance. While pericardial delivery of antiarrhythmics and cardioprotective

treatments hold great promise, dosing and timing will need to be optimized for the

clinical scenarios. The DHA used in this study was dissolved in ethanol, future studies

may try to make use of a formulation that is not dissolved in a substance that is known to

increase heart rate and decrease contractility [30].

Chapter 6: Effects of pericardial treatment with polyunsaturated fatty acids, electrophysiology and global ischemia

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Preface

In Chapter 6, I continued the work into ω3PUFAs. In the previous chapter, the DHA that

is used is dissolved in ethanol, which has competing cardiac effects of its own. For

chapter 6, in addition to using an alternative ω3PUFA formula, Omegaven, steps were

also taken to simplify the clinical procedure. In other words, the DHA used in chapter 5

was in a chemical formulation that would require additional approval for clinical use. The

path to approval for IP delivery may be simplified by using formulas that have been

proven safe for IV infusion, such as Omegaven, which is used clinically as a fatty acid

emulsion for parenteral nutrition.

Introduction

The delivery of omega-3 polyunsaturated fatty acids (ω3PUFA) to the pericardial space holds great promise. The antiarrhythmic and ischemic protection have been demonstrated in a swine model of acute myocardial infarction [1]. We have also show the potential for reducing atrial fibrillation (AF) in previous studies.

The ω3PUFA that was used in previous study was in its free form, but dissolved in ethanol. In this form, it is not clinically approved for injection, and would be difficult to translate it to clinical studies. If beneficial effects can be seen in a formulation that has been approved for clinical use, it would be simpler to get approval since the formula has been proven safe for injection.

Several fatty acid emulsions are approved for parenteral nutrition. Mostly used in patients who have digestive abnormalities, these emulsions are administered intravenously (IV) to provide nutrition. As such, they have been proven safe and effective for injection. Thus minimal complications exist with myocardial tissue at recommended levels, and intrapericardial delivery would have precedence to cite when getting approved for investigational use and potentially for on label use.

While the previously tested ω3PUFA was pure DHA, the available emulsions are mixtures of ω3PUFAs and ω6PUFAs. The three compounds used in this experiment are outlined below in Table 6.1. We hypothesize that the Omegaven treatment, which is an emulsion primarily made up of ω3PUFAs, will lower time in AF and improve hemodynamic function *in vitro*, while the primarily omega-6 compounds will have almost

no effect on AF time, but may exert a slight depression in hemodynamics due to the increase in pro-inflammatory signaling.

Omegaven	Lipovenos	Intralipid
50% Omega-3 -20% EPA -23% DHA	4% Omega-3	8% Omega-3
10% Omega-6	22% Omega-6	45% Omega-6
10% Omega-9	9% Omega-9	18% Omega-9
25% Glycerol	42% Triglycerides	5% Phospholipids
Saturated Fatty Acids	6% Phospholipids	10% Glycerol
	10% Glycerol	Saturated Fatty Acids
	Saturated Fatty Acids	

Table 6.1: Cocktail treatments composed of omega-3 and omega-6 PUFAs

Methods
Surgical procedure

A total of 36 healthy, castrated male Yorkshire swine, weighing 87±9kg, were used for this study. Anesthesia and instrumentation were set up as described in chapter five, with the exception of transmural and His bundle electrophysiology catheters. Endocaridal pacing leads (Medtronic 3830, Medtronic, Minneapolis, MN) were secured into the right ventricular apex and right atrium via fluoroscopy (see Figure 5.) and connected to a GEM III pacemaker/defibrillator (Medtronic, MN). After medial sternotomy, access to the heart was then attained via a medial sternotomy and a pericardial cradle was created (Figure 5.).

Electrophysiologic measurements

The electrical leads were used to measure refractory periods and susceptibility to AF. The signals from the pacemaker programmer (Medtronic 2090, Medtronic, MN),

epicardial pacing leads, decapolar catheter, and EKG leads were recorded

simultaneously by an ENSITE electrophysiology system (St. Jude Medical, MN).

Atrial effective refractory periods (AERP), ventricular effective refractory periods (VERP),

and atrioventricular nodal effective refractory periods (AVNERP) were measured

following a standard protocol explained by Ayers et al. [2] and outlined in chapter 5.

These electrophysiology parameters were taken just prior to drug delivery as well as at

5, 30, 60, and 90 minutes after drug delivery.

After the initial data collection, the heart was given one of three treatments in the

pericardial space: Omegaven, Lipvenos, Intralipid (Fresenius Kabi, Hamburg, Germany).

All treatments infused at a constant rate 0.5mL/min for 30 minutes for a total of 15mL.

In vitro performance

At the end of each treatment, the heart was arrested and excised as described in

chapter 5.   The great vessels were cannulated, and the heart was reperfused with krebs

buffer using Visible Heart® methodologies (Figure 5.) [3]. Hemodynamic data were

collected throughout the first four hours of reanimation as described in chapter 5. The

krebs perfusate was tested for Troponin-I and Myoglobin tested at the Fairview hospital

clinical labs, and TBARS ELISA assays (Cayman) were also performed. As the heart

was cycled in and out of working mode, short axis ultrasound images were recorded in

order to classify myocardial edema.

Statistics

Data are presented as mean with standard deviation. Significance was determined

using Student's t-test or ANOVA, where appropriate. An α of 0.05 was considered

significant, with bonferroni corrections when appropriate. Analysis was done using

Microsoft Excel 2007 and STATA version 10.1

Results

36 pigs were used in this study with no major morbidity or mortality detected from the

treatments. There were no significant differences in animal weight or heart weight

among groups (Table 6.2). No differences exist among the groups in cold ischemia

time, defined as the time between arrest and defibrillation to start reanimation.

Treatment	n	Animal wt (kg)	Heart Wt (g)	cold ischemia time (min)
Saline	9	85.1±8.7	418.8±43.9	72.3±5.8
Omegaven	9	88.9±8.6	433.6±40.8	67.2±6.1
Intralipid	11	92.6±10.3	459.1±57.9	74.9±10.9
Lipovenos	7	83.4±5.8	425.0±48.8	76.5±11.0

Table 6.2: Baseline characteristics, including animal weight, heart weight and cold ischemia time.

During the explant procedure, time from cross clamp to cessation of electrical and

mechanical activity was recorded as time to flatline. Upon reanimation, the number of

shocks required to restart the heart was also recorded (Table 6.3).

Tx	n	Time to flatline (sec)	number shocks to restart
Saline	9	61±44.0	4.3±4.1
Omegaven	9	72.6±35.0	4.4±5.4
Intralipid	11	53.7±29.5	2.9±2.0
Lipovenos	7	51.8±24.8	3.8±3.9

Table 6.3: Time to flatline upon cardioplegia administration and the number of defibrillation shocks required to restart the heart.

Atrial fibrillation

In this open chest model, pig hearts were induced into atrial fibrillation with a burst pace. The saline control hearts had very little change from baseline. The Lipovenos treated hearts reduced the time in AF from baseline, with significant differences seen at 5, 30 and 60 minute, as well a significant reduction from saline treated hearts at the five minute time point (Figure 6.1).
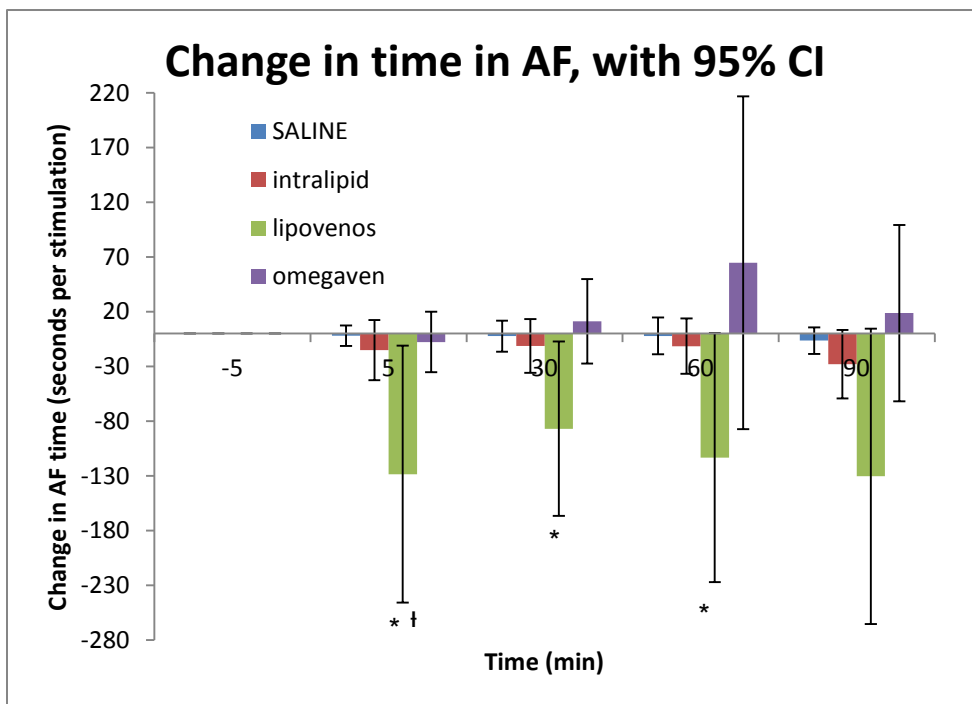
Figure 6.1: Change in time in AF from baseline. There was significant reduction in AF from baseline in the Lipovenos treated hearts. The * indicates significant change over baseline; ǂ indicated significant reduction over saline treatments

Refractory periods

Though most of the treatments made ventricular effective refractory periods (VERP) trend slightly upwards, there were no significant differences among the groups or significant changes from baseline. Atrial effective refractory periods (AERPs) stayed within 10ms of their starting values, with no statistically significant changes (Figure 6.2).
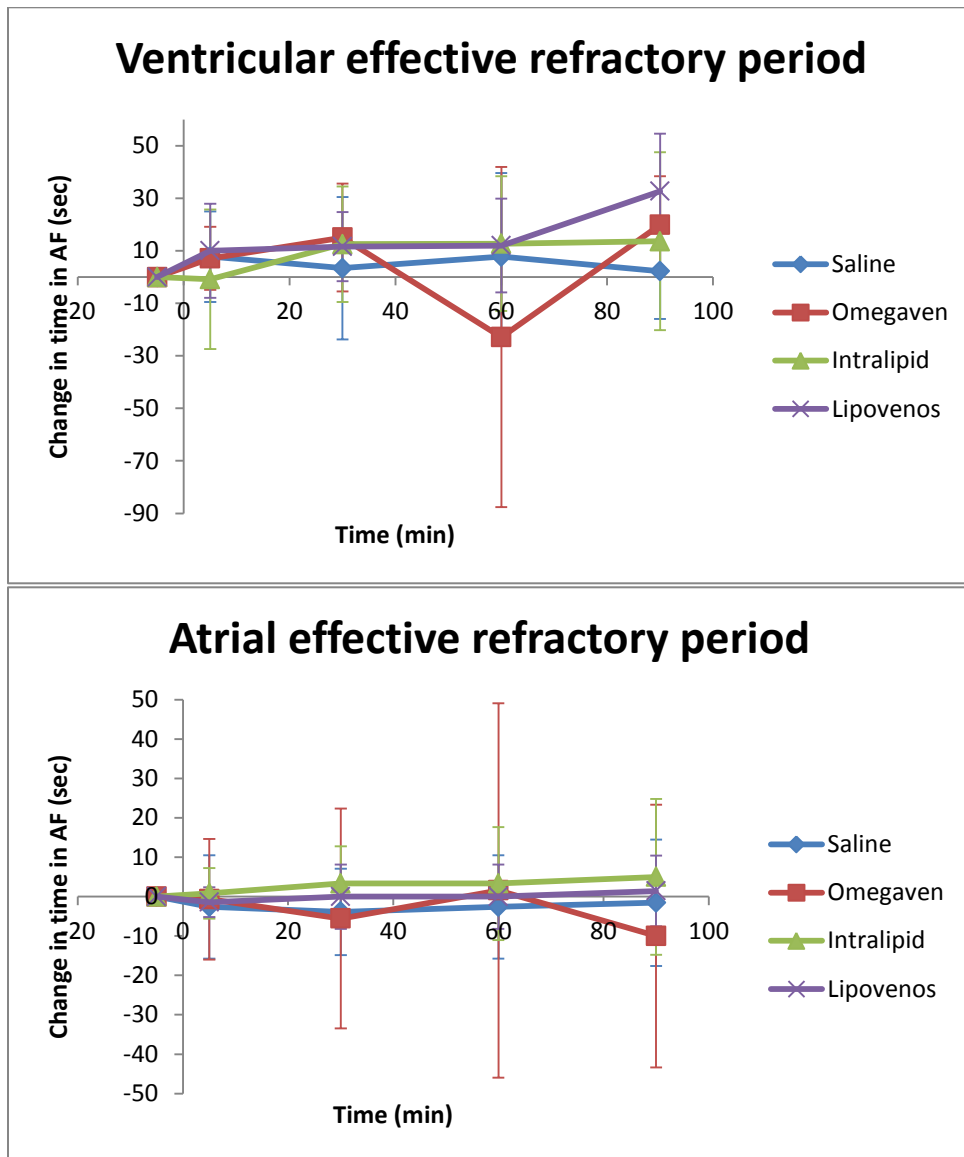
Figure 6.2: Atrial and ventricular effective refractory periods.

Hemodynamics

The Lipovenos treated hearts lowered heart rate at 5 and 30 minutes (compared to saline), and maintained a slight reduction in heart rate for the duration. The heart rate for the saline and Intralipid treated hearts increased slightly from baseline, but not a statistically significant amount (Figure 6.3).
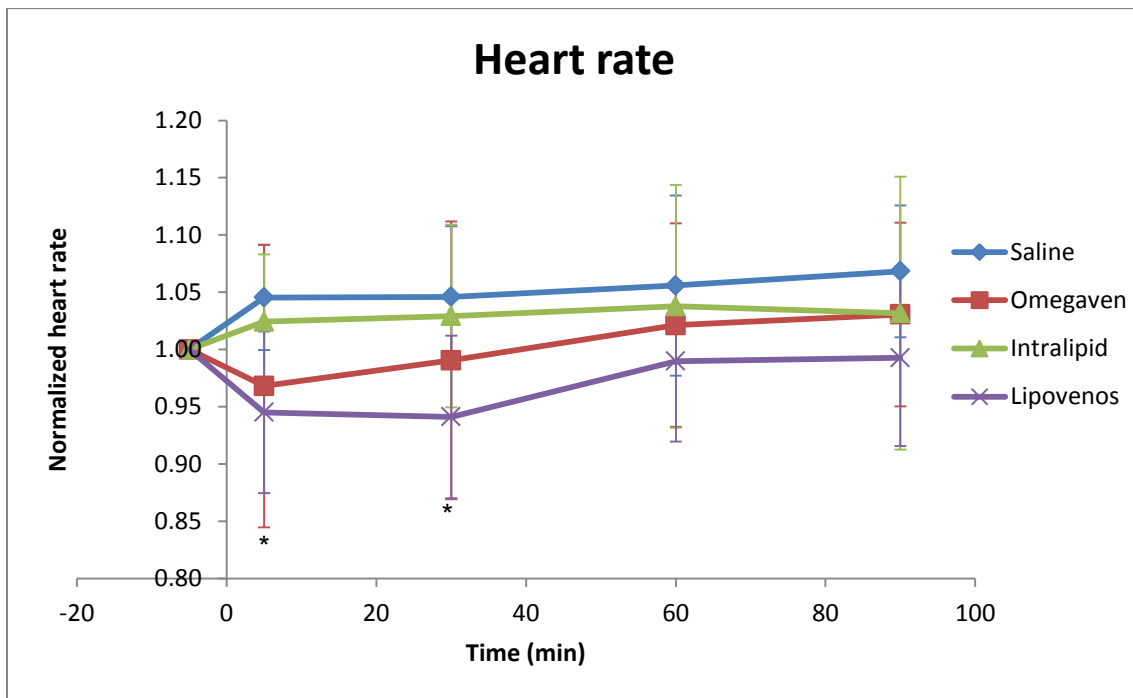
Figure 6.3: Heart rate was slightly lowered in the Lipovenos treated hearts at 5 and 30 minutes when compared to saline.

Left ventricular pressures

Both during treatment administration and upon reperfusion, the pressures were recorded. The maximum and minimum LV pressures were measured both *in vivo* and *in vitro* on the Visible Heart® apparatus.

Maximum LVP did not vary significantly during the in vivo administration, however, there

was a 12-17% increase upon reanimation in all groups. There were minimal differences

among the groups for the first hour (Figure 6.4. However, as pressure was tracked

through the four hours of reanimation, the PUFA treated hearts maintained steady

pressures, but the function of the saline treated hearts started to decline after two hours.
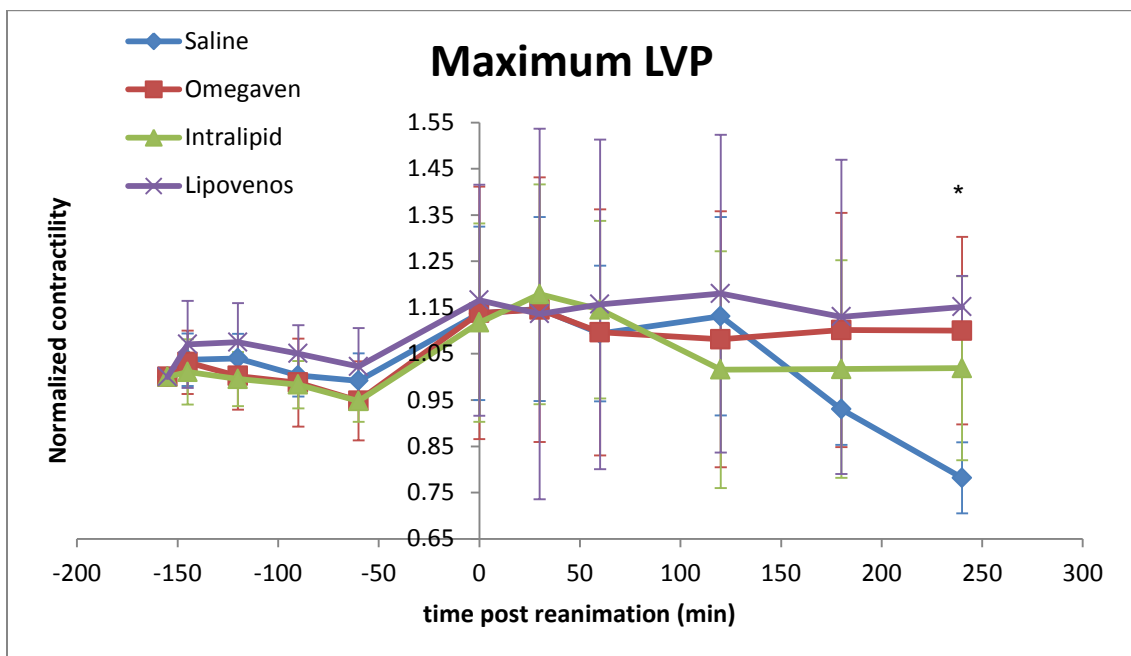
Figure 6.4: Maximum left ventricular pressure stayed steady throughout most of the four hours in vitro for the three treated groups. However the saline treated group began to contract with less force after two hours, with significant differences between Omegaven and Lipovenos seen at the four hour data point.

Minimum left ventricular pressure was also calculated Figure 6.5. The hearts started

with a minimum LVP at 5mmHg, with no large changes in vivo. Upon reanimation, there

was a slight increase of 1-5mmHg. During the course of reanimation, the minimum

pressure continued to increase, with both Omegaven and Lipovenos treated hearts

having significantly lower pressures compared to saline by the end of four hours.
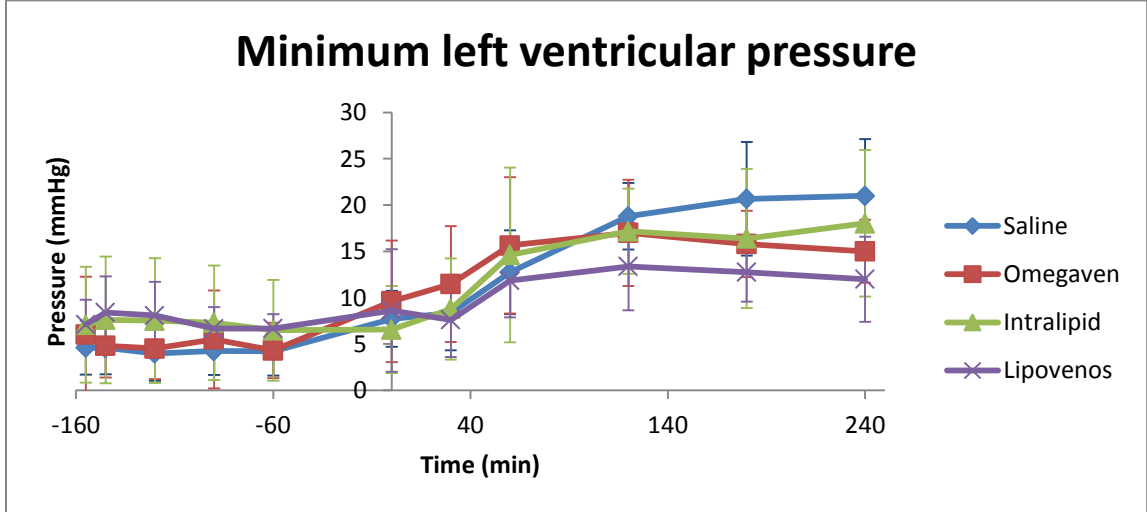
Figure 6.5: Minimum left ventricular pressure. The minimum LVP slowly increases over time for all groups.

Protein levels

The levels of biomarkers measuring cell injury were also collected. There was an

observed buildup in levels of lactate, troponin and myoglobin over the course of the first

50 minutes of reperfusion (Figure 6.6)
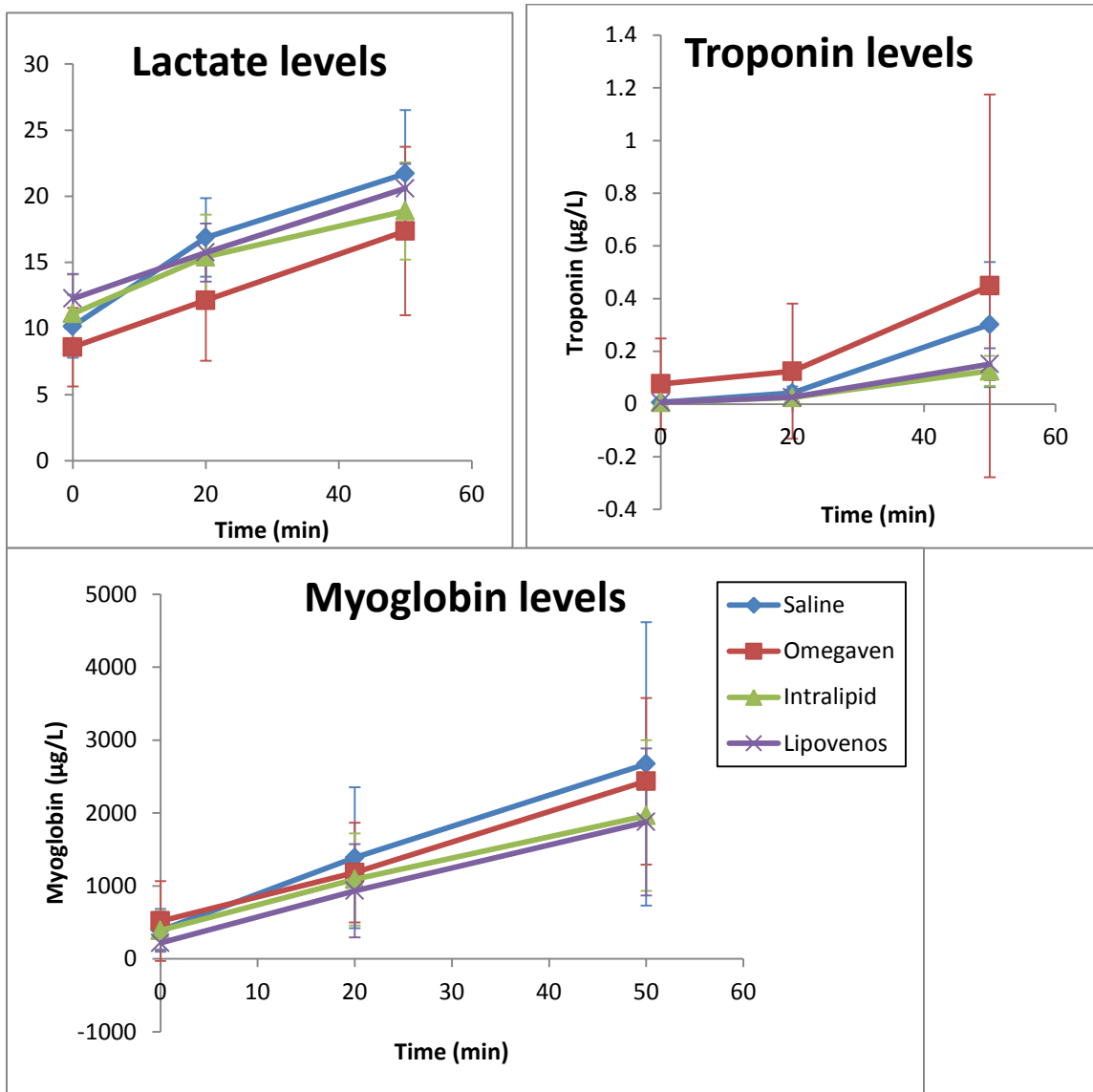
Figure 6.6: Biomarkers detected in the circulating krebs buffer. The levels of each continued to rise throughout the first hour of reperfusion, where there is little difference among the groups.

Ultrasound

In order to measure the myocardial edema, short axis echocardiographic images were

captured throughout the four hours of reperfusion. The size was traced out on the

ultrasound unit with the imbedded analysis software (GE, Fairfield, CT). Myocardial

cross-sectional area was normalized to the area in right-sided working mode. There

were slight increasing trends for all groups, with no significant differences (Figure 6.7).
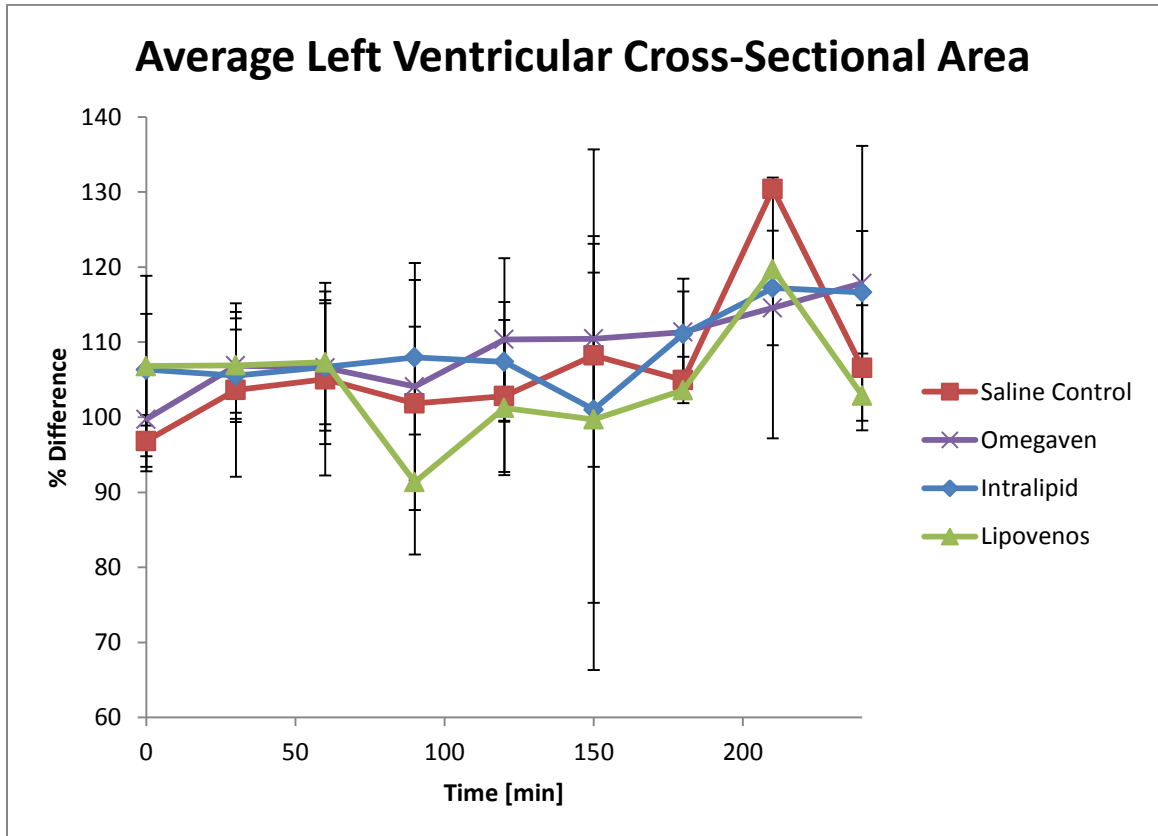
Figure 6.7: Left ventricular cross-sectional area as measured on short axis ultrasound. All treatments increased slightly, with no significant differences.

Discussion

In this study we infused three different fatty acid emulsions into the swine pericardial

space to test their ability to reduce susceptibility to induced AF and improve

hemodynamic function upon reanimation. In this model, an infusion of Lipovenos

significantly reduced time in induced AF both from baseline levels and compared to the

saline controls (Figure 6.1). None of the other treatments had significant effect on AF time, with the exception of a non-significant increase in AF time at 60 minutes for the Omegaven treated animals. This spike is primarily caused by a single outlier at this time point. If excluded from the analysis, the Omegaven time in AF would remain very close to zero.

The heart rate of all groups stayed within 10% of baseline during in vivo portion of the experiment. The Lipovenos treated hearts had a significant reduction in heart rate during the period of time in which the treatment was being infused (5 and 30 minutes); however the difference was reduced after the infusion ended (60 and 90 minutes).

Upon reanimation, there was a slight jump in maximum left ventricular pressure in all groups. Previous studies of performance in our lab have shown that heart function declines on the apparatus [3]. In the present study, the max LVP for the treated hearts maintained 90-100% of function throughout the four hours measured in this study, while the saline control group lost over 30% of its initial in vitro function in the same time period.

Similarly in the minimum left ventricular pressure, values are expected to increase as the heart beats outside of the body and is mildly ischemic. While the Lipovenos treated hearts increased 30-55% over in vitro baseline throughout reanimation, saline controls increased nearly three-fold, with significant differences for the last two hours.

While Omegaven is made up of a large percentage of omega-3 fatty acids, and Intralipid contains a large percentage of omega-6 fatty acids, Lipovenos is somewhere in the middle of these two. The main component of Lipovenos that not present in the other two forms is a high concentration of triglycerides. Most fatty acids in the body are stored in triglyceride form, and supplying treatment in this way may deliver them in a form such that they are readily available.

The significant reduction in AF was only seen for the first 60 minutes, but not the final 90 minute data point. This may be because the delivery was complete and the diffusion of fatty acids out of the myocardium was greater than the rate going in. However, it is important to note that there was still an improvement in the in vitro function compared to saline four hours after the treatment had ended. This may be due to a higher concentration of fatty acids to elicit antiarrhythmic effects, while the protective effects are expressed at lower concentrations

One advantage of using these fatty acid emulsions in the present formulation is they have been approved for clinical use, and proven safe infused into the venous system. The treatment infusion in this study lasted for 30 minutes, with some indication of reduced effects upon termination, as seen in the AF time and heart rate. This indicates the potential quick action of these agents, which may not require a full 90 minutes to diffuse. Thus future studies are designed to evaluate effects of shorter infusions. Such shorter infusions would also be more readily adopted into cardiothoracic procedures.

Conclusions and future work

Using clinically approved fatty acid emulsions may reduce hurdles to clinical adoption.

Here we showed that the nutritional supplement Lipovenos can reduce AF and improve

hemodynamics when administered to the pericardial space. This may have significant

impact in cardiac transplant. Future work will include additional dose-response and

infusion rate testing. Tailoring delivery to cardiac surgery may involve reduced infusion

times, but may improve outcomes in the over one million cardiothoracic surgeries each

year.

Chapter 6b: Effects of short pericardial treatment with polyunsaturated fatty acids, electrophysiology and global ischemia

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Preface

The experiments in this chapter were performed concurrently and using the same protocol at in chapter 6. The results are divided out here to improve clarity. The main parameter that was adjusted in this chapter was that instead of the 90 minute therapy, a 30 minute treatment was tested. While the original application for the pretreating to improve outcomes after heart transplantation, where a 90 minute window is feasible, pretreating for that length of time before routine cardiac surgery would not be practical. After getting feedback from physicians here at the University of Minnesota Fairview hospital, it was decided that a 30 minute treatment was much more acceptable when time was limited. For example, such a treatment could be given during preparation for bypass.

Introduction

The delivery of omega-3 polyunsaturated fatty acids (ω3PUFA) to the pericardial space holds great promise. The antiarrhythmic and ischemic protection have been demonstrated in a swine model of acute myocardial infarction [1]. We have also show the potential for reducing atrial fibrillation (AF) in previous studies.

The 90 minute treatment used in previous studies allows ample time for the fatty acid to diffuse into the myocardium, but may not be ideal for all clinical applications. A 30 minute pretreatment would be much more suitable for most cardiothoracic surgeries. For instance, a pericardial infusion could begin while a surgeon is preparing the internal thoracic artery for coronary artery bypass grafting. Such a window may allow for 20-40 minutes of treatment while the pericardium in mostly intact. A shorter treatment time also decreases the time pericardial access is required. This can reduce the risk of infection [2], [3]. Decreased treatment time also decreases the time in the operating room, decreasing procedure cost.

Methods
Surgical procedure

A total of 29 healthy, castrated male Yorkshire swine, weighing 83±7.8kg, were used for this study. Anesthesia, instrumentation and measurements were taken as described in chapter 6. For these animals, the heart was given one of three treatments in the pericardial space: DHA, Omegaven, or control with saline. All treatments infused at a constant rate of 1mL/min for 15 minutes. All hemodynamic and electrophysiological

measurements were taken for 30 minutes after drug delivery after which the heart was removed

In vitro performance

As in chapters 5 and 6, after excising the heart, it was reperfused with krebs buffer and reanimated using Visible Heart® methodologies [4]. Hemodynamic and biomarker data were collected as described previously.

Statistics

Data are presented as mean with standard deviation. Significance was determined using Student's t-test or ANOVA, where appropriate. An α of 0.05 was considered significant, with bonferroni corrections when appropriate. Analysis was done using Microsoft Excel 2007 and STATA version 10.1

Results

A total of 29 pigs were used in this study with no major morbidity or mortality detected from the treatments. There were no significant differences in animal weight or heart weight among groups (Table 6b.1). No differences exist among the groups in cold ischemia time, defined as the time between arrest and defibrillation to start reanimation.

Treatment	n	Animal wt (kg)	Heart Wt (g)	cold ischemia time (min)
Omegaven	10	80.3±8.8	395.8±39.8	77.7±9.4
DHA	9	84.8±9.0	420.4±33.4	79.7±7.3
Control	10	85.1±8.0	440.9±52.4	78.7±24.6

Table 6b.1: Baseline characteristics, including animal weight, heart weight and cold ischemia time.

During the explant procedure, time from cross clamp to cessation of electrical and

mechanical activity was recorded as time to flatline. Upon reanimation, the number of

shocks required to restart the heart was also recorded, with no significant differences

observed (Table 6b.2).

Treatment	n	Time to flatline (sec)	number shocks to re1start
Omegaven	10	97.8±57.6	1.56±0.73
DHA	9	58.1±25.8	2.0±1.31
Control	10	54.8±14.6	2.43±2.38

Table 6b.2: Time to flatline upon cardioplegia delivery and the number of defibrillation shocks required to restart the heart.

Atrial fibrillation

In this open chest model, pig hearts were induced into atrial fibrillation with a burst pace.

None of the treatment groups changed from baseline in this short treatment (Figure

6b.1).
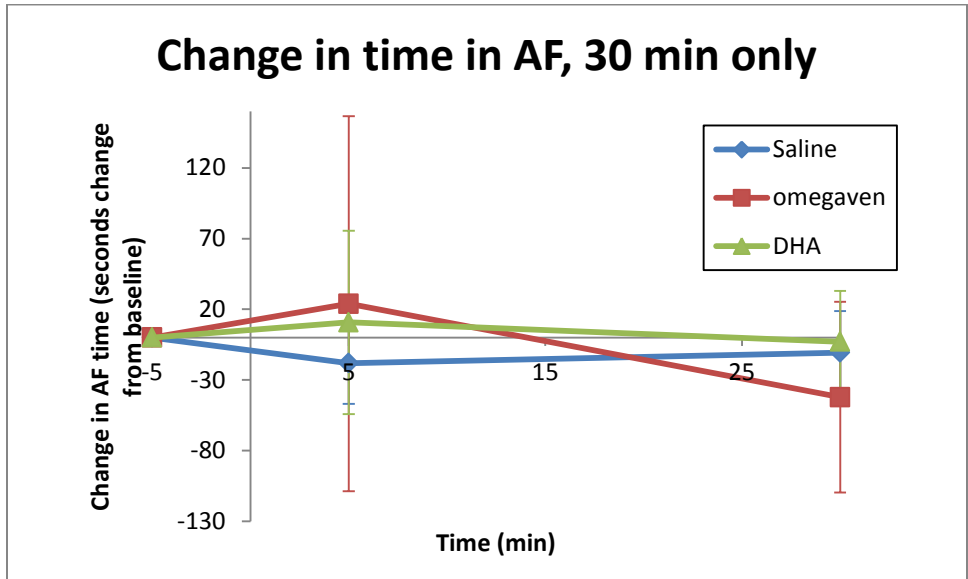
Figure 6b.1: change in time in AF from baseline values.

Refractory periods

There were no significant differences among the groups or significant changes from baseline in either the atrial effective refractory periods (AERPs) stayed within 20ms of their starting values, with no statistically significant changes. The ventricular effective refractory periods (VERPs) of all groups stayed within 5ms of baseline, with no significant changes (Figure 6b.2).
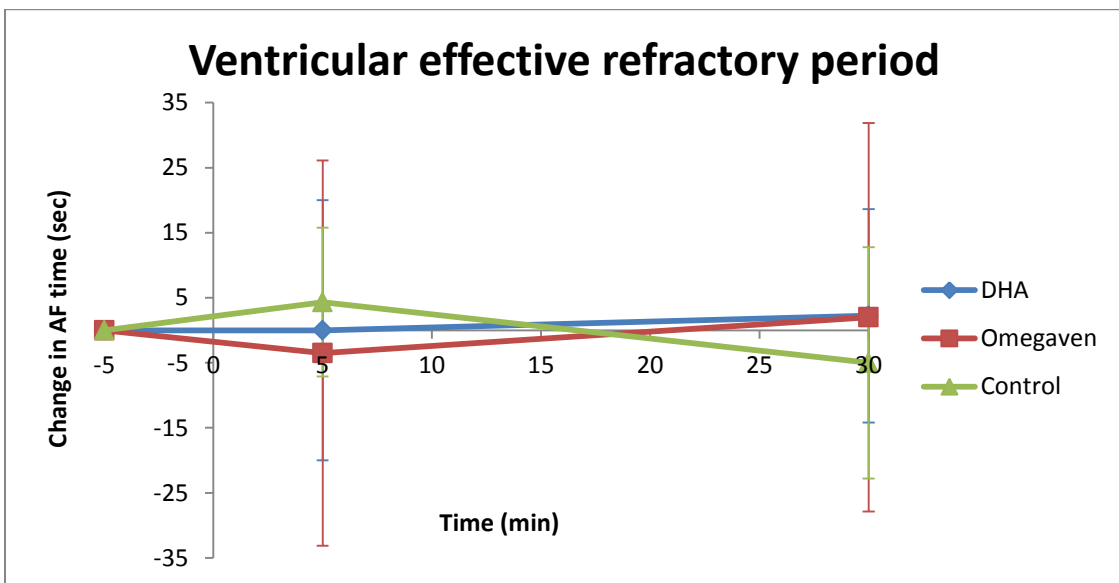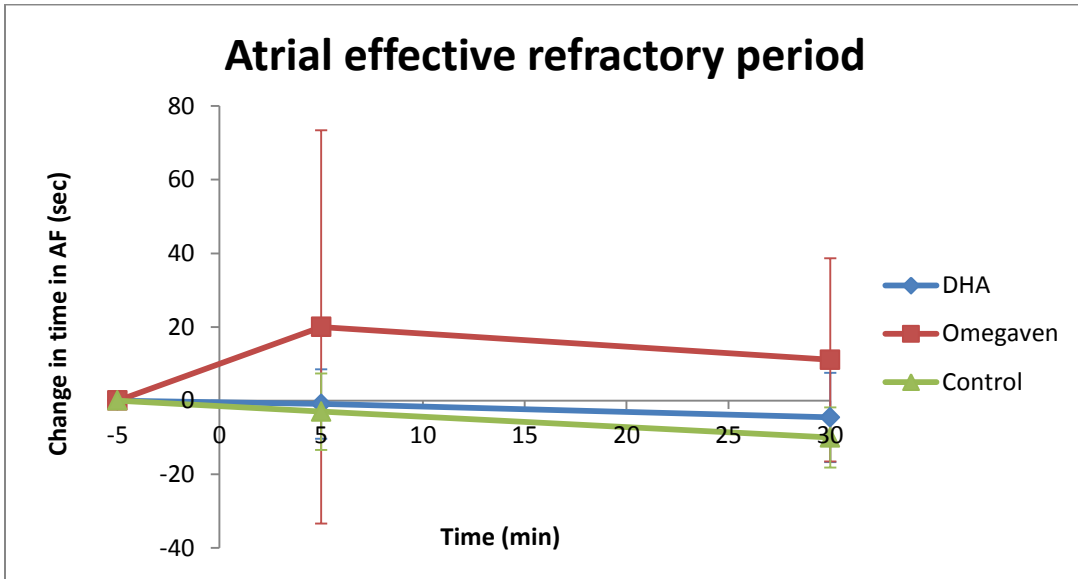
Figure 6b.2: Atrial and ventricular effective refractory periods. Shown as change from baseline refractory period.

Hemodynamics

The heart rate was measured in for each treatment group. There was a slight increase in heart rate for all groups, though no significant differences were observed (Figure 6b.3).
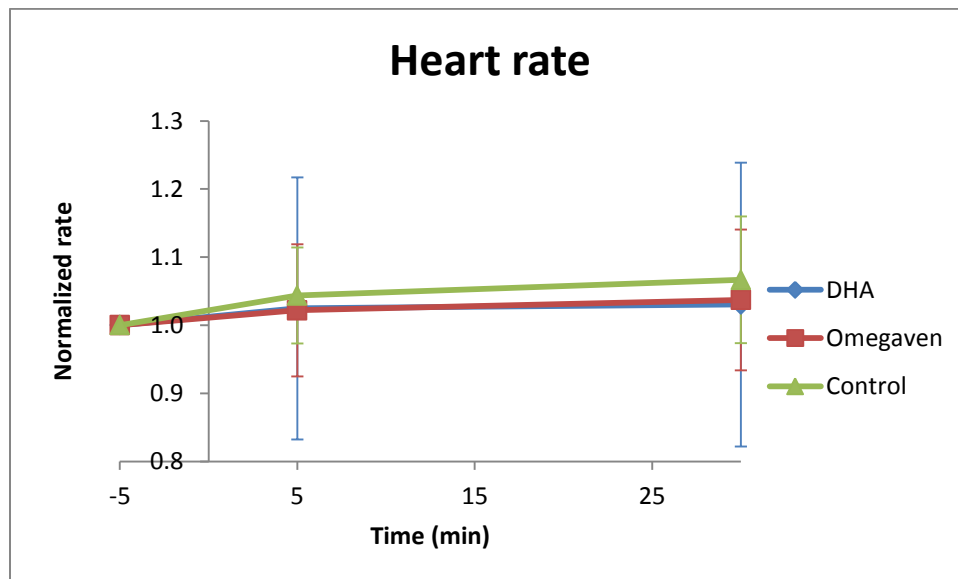
Figure 6b.3: Heart rate in for three treatment groups remains nearly constant.

Left ventricular pressures

Prior to heart explant, there was minimal change in blood pressure. Upon reanimation, the maximum left ventricular pressure (LVP) jumped 22% in the DHA group. The DHA treated hearts had significantly higher maximum LVPs at several points throughout the four hours of reanimation. All groups had stable maximum LVPs for the first two hours, followed by slight decline. The saline treated hearts had the lowest maximum pressure, which also declined to the lowest value in the last two hours (Figure 6b.4).
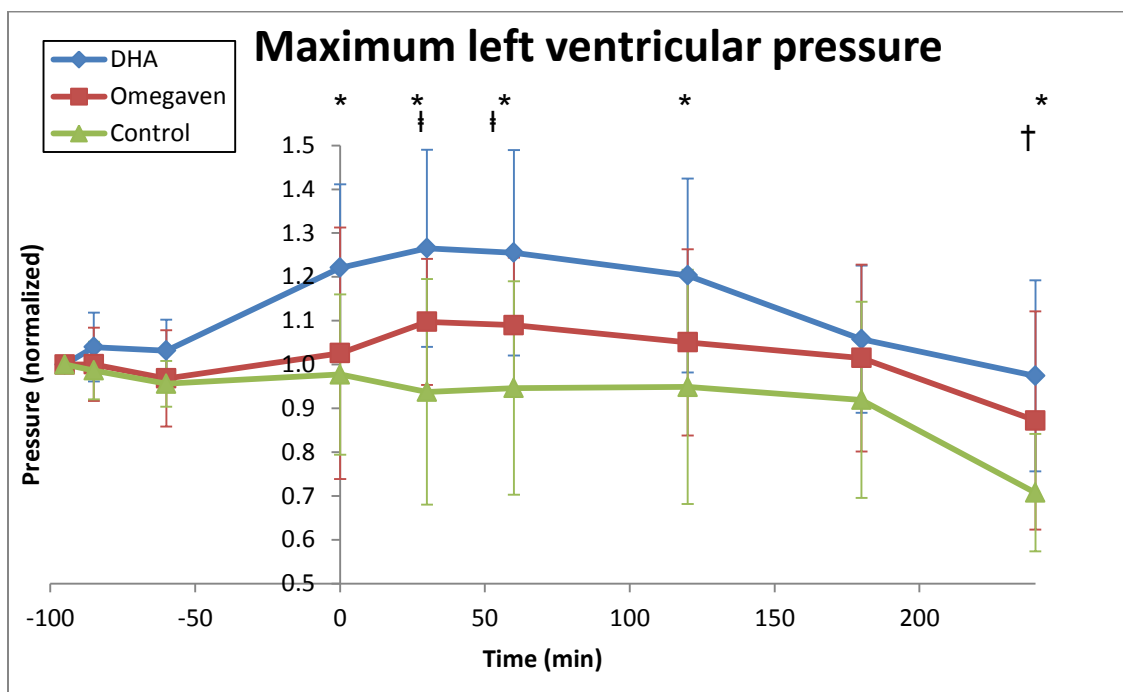
Figure 6b.4: Maximum left ventricular pressure. The DHA treated hearts held significantly higher function compared to saline controls at several points during reperfusion (*). The DHA treated hearts also contracted with more pressure compared to the Omegaven treated hearts at the 30 and 60 minute data collection points (ǂ). The Omegaven treated hearts had higher maximum pressure compared to saline, with a significant difference only found at the four hour collection point (†).

Minimum LVP was also tracked throughout the experiment. There were

indistinguishable differences among the three groups, which all rose in pressure

throughout reperfusion (Figure 6b.5).

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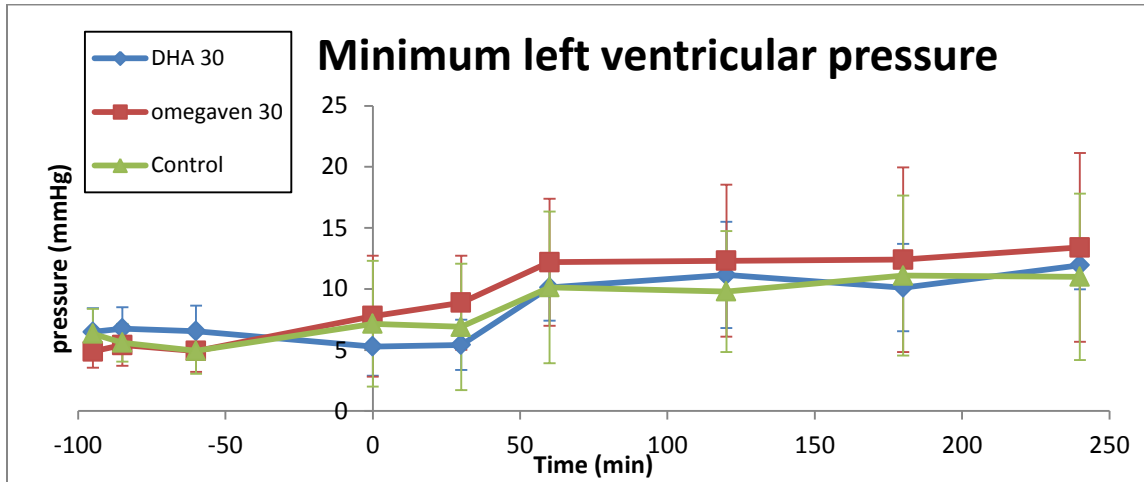
Figure 6b.5: Minimum left ventricular pressure. No significant differences were observed among the three groups.

Protein levels

The levels of biomarkers measuring cell injury were also collected. There was an

observed buildup in levels of lactate, troponin and myoglobin over the course of the first

50 minutes of reperfusion (Figure 6b.6).

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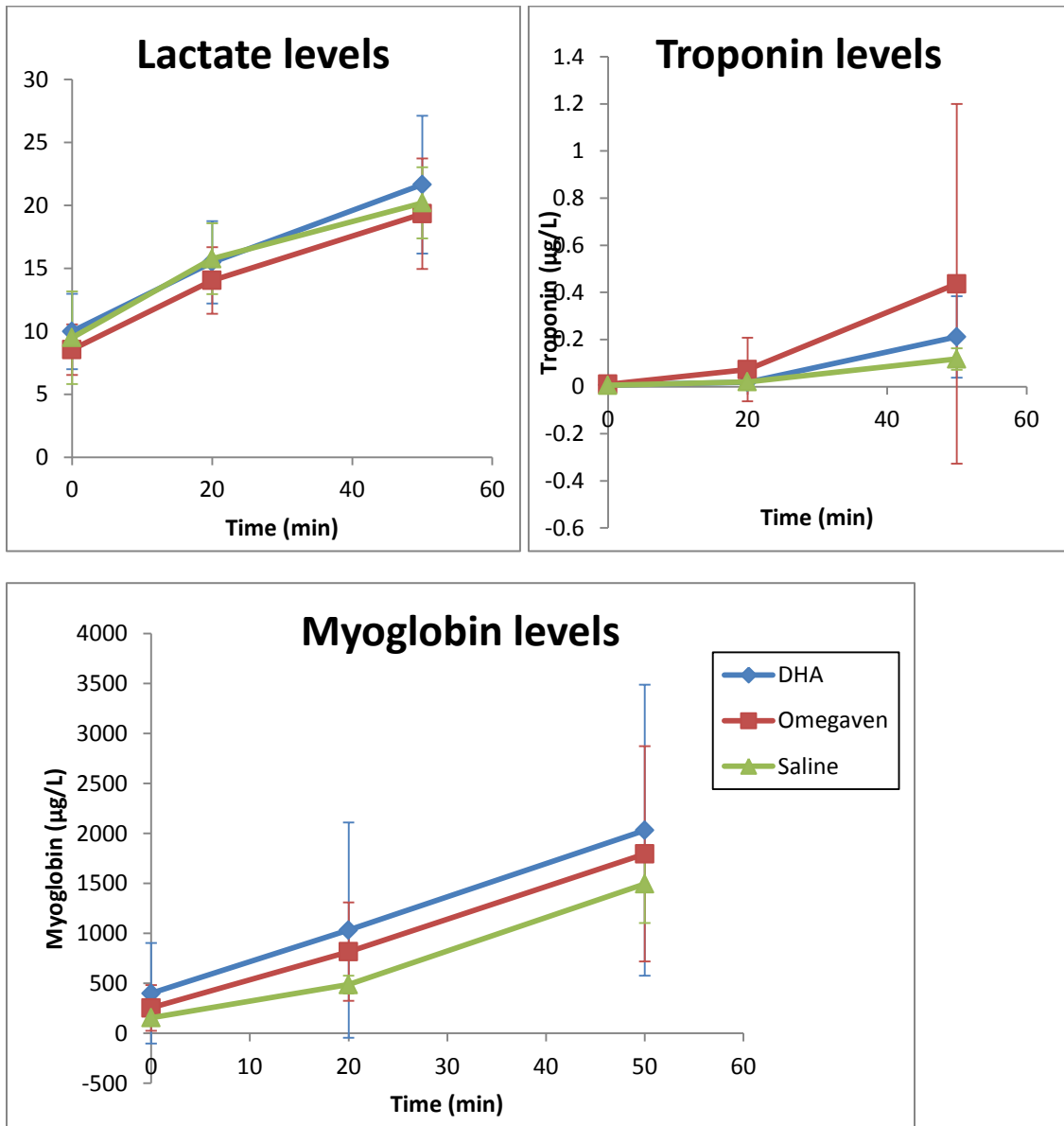
Figure 6b.6: Biomarkers detected in the circulating krebs buffer. While the levels of each continued to rise, there is little difference among the groups for each biomarker.

Discussion

With over a million cardiac procedures in the US per year, any improvement in outcomes

or reduction in complications would have significant impact on improving patient heath

and lowering healthcare cost. A simple pericardial treatment while the surgical site was being prepared would add minimal time, cost or complication to many cardiothoracic procedures. Previous studies have shown pretreatment with DHA provides excellent antiarrhythmic and cardioprotecive effects during acute ischemia in swine[1].

In vivo, there was little impact by any of the treatments. Previous doses of DHA (chapter 5) showed non-significant increase in VERPs and a significant reduction in time in AF. The main difference was the previous treatments started with a loading dose of DHA followed by a steady infusion. With the thought of simplifying the protocol, the current procedure eliminated the loading dose. The resulting effect was almost no response in the refractory periods or time in AF observed in the current study. Thus it may be necessary for higher doses of ω3PUFAs to be administered before electrophysiological effects can be observed.

There were, however, significant hemodynamic improvements observed with DHA infusion, as well as non-significant improvements in Omegaven treated hearts. These improvements with ω3PUFAs may be a result of the anti-inflammatory effects [5], [6] as well as anti-apoptotic effects [7]. In addition, ω3PUFAs have been shown to help decrease oxidative injury [8]. These effects likely combine to reduce cell damage and death, contributing to the cardioprotective properties of PUFA.

Limitations

Although the Visible Heart® approach provides an excellent model for studying cold ischemia and heart function, there are several limitations. Because the circulating buffer

has no oxygen carriers, the working heart undergoes minor global ischemia. It is thus

necessary to cycle the heart out of a full working mode into a "resting" right side working

mode. In this mode the right side of the heart is pumping little more than the coronary

effluent. It is also important to note that a transplanted heart would have reduced neural

connectivity, but it would maintain hormonal responses. The tissue in the in vitro portion

of this experiment lacks all neurohormonal influences from the body. While this differs

from the in vivo conditions, the Visible Heart$^®$ setup makes it possible to isolate cardiac

responses from other influences.

Conclusions and future work

We have shown here that an infusion of the ω3PUFA DHA can improve hemodynamic

function after global ischemia and reperfusion. Previous experiments with variations in

delivery protocol have shown slight variations of effect. Clearly, the dose, delivery rate,

timing and form of ω3PUFAs impact the cardiac effects. Future work includes

refinement of delivery protocol and, ultimately, clinical trials to determine effectiveness in

humans.

Chapter 7: Influence of Supplemental Lipids on Hemodynamic and Metabolic Performance of the Isolated Swine Heart

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Preface

One of the most common types of ischemia comes as a result of acute myocardial infarctions. Because such an event is often unpredictable, pretreatments are of limited use. To test the effectiveness of fatty acids as a post-treatment therapy, we used a similar protocol as with the previous *in vitro* studies. Instead of pretreating the animals prior to explant, the hearts were treated upon reanimation in the Visible Heart apparatus. And while preconditioning with fatty acids has been observed to minimize damage from ischemia/reperfusion injury [1], [2], the mechanisms are not clear. *Chapter 7: Influence of Supplemental Lipids on Hemodynamic and Metabolic Performance of the Isolated Swine Heart* provides a summary of the hemodynamic measurement after administrations of two different fatty acid formulas during reperfusion of swine hearts.

Summary

Background: In healthy hearts, free fatty acids (FA) are the preferred myocardial metabolic substrate. Further, certain classes of FAs have been shown to exhibit antiarrhythmic and other cardio-protective properties. The current study utilized isolated swine hearts to examine the cardiac effects of an omega-3 polyunsaturated FA (Docosahexanoic acid; DHA) and a commercially available triglyceride mixture (Intralipid 20%; IL). We hypothesized that lipid compounds would increase hemodynamic performance, with speculation that DHA would provide a greater improvement.

Methods: Yorkshire swine (n=40) were anesthetized and hearts were excised via median sternotomy. Hearts were then cannulated and reperfused utilizing the Visible Heart® methodology. Prior to reanimation, hearts were instrumented for left ventricular pressure (LVP) measurement. In vitro, hearts were separated into DHA, IL, and control groups and hemodynamic data were collected during each of three perfusion modes (Langendorff, right-side working, and full-working) over the course of an hour.

Results: Hemodynamic benefits from lipid treatment were most evident during full-working mode. Treatment with IL resulted in significantly higher ($p<0.05$) maximum LVP at several points during the first hour when compared to control, and higher positive LVP time-derivative (dP/dt_{max}) compared to DHA and control. IL also resulted in significantly lower ($p<0.05$) minLVP than DHA and control, and lower dP/dt_{min} than control.

Conclusions: Lipid administration conferred a level of cardioprotection manifested as improved systolic and diastolic functioning. It is interesting to note, however, that IL resulted in greater improvement compared to DHA. It is likely that performance enhancement with IL was maintained due to mechanisms other than metabolic.

Introduction

Under normal physiologic conditions, cardiac muscle utilizes high-energy fatty acids (FA) as its primary metabolic substrate. However, during heart failure or following myocardial infarction, FA metabolism is disrupted, causing a shift to a glucose-based metabolism [3–5]. Preconditioning with FA substrates has been shown to minimize subsequent ischemic damage, and also to minimize arrhythmias [1], [2]. Even so, the underlying cardiac mechanisms responsible for the benefits provided by circulating free FAs are not clear. By isolating the heart from the systemic influences present in vivo, it is possible to investigate specific cardiac effects of FA administration.

The current study used the global ischemic condition of the isolated heart preparation to investigate hemodynamic and metabolic effects of administering Intralipid 20% (IL; Sigma-Aldrich, St. Louis, MO, USA) or docosahexanoic acid (DHA; Cayman Chemical Co., Ann Arbor, Mi, USA) following an ischemic event. In particular, the isolated swine heart maintained with a crystalloid perfusate can be considered globally ischemic; our lab has utilized this model extensively for a number of scientific purposes [6–9]. Such isolated hearts are able to produce intrinsic electrical activation or can be paced [10]. It is generally accepted that swine hearts are anatomically and electrophysiologically similar to human hearts, particularly concerning arrhythmia potential [11], [12] With this in mind, we hypothesized that the addition of lipid compounds would better preserve hemodynamic function of the isolated heart preparation when compared to control hearts. We further hypothesized that DHA would improve hemodynamics to a greater extent than IL.

Methods

The research protocol was reviewed and approved by the University of Minnesota Institutional Animal Care and Use Committee, and was designed to ensure the humane treatment of all animals as outlined by the "Guide for the Care and Use of Laboratory Animals" (NIH). There was no practical difference in the time of day for each of the experiments performed and all animals were handled in a similar manner prior to investigation (acclimated for a week prior to the study and fasted for 24 hours). All data were collected using a Windows-based analog-to-digital data acquisition system running the IOX software (EMKA Technologies, Paris, France).

In situ protocol

Yorkshire-cross mongrel swine (n=40, 82.9 ± 9.4 kg) were initially anesthetized using intravenous midazolam (2-3 mg/kg), then intubated and mechanically ventilated to allow administration of isoflurane to maintain a surgical depth of anesthesia (>1.0 MAC). Catheter access to the right and left ventricles (RV, LV) of the heart was achieved via incisions in the external jugular vein and right common carotid artery, respectively. Separate Millar Mikro-Tip® pressure catheters (5 French (Fr), MPC 500, Millar, Houston, TX, USA) were inserted into the RV and LV so to allow for continuous pressure assessment. Following internal instrumentation and closed-chest hemodynamic data collection, a medial sternotomy was performed, exposing the heart.

Isolation and in vitro protocol

Upon completion of in situ data collection and intravenous heparinization (300 IU/kg), hearts were arrested using refrigerated (4°C) St. Thomas' cardioplegia, then excised and

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cannulated for attachment to the Visible Heart® apparatus following a protocol similar to that previously described [6]. The excision and cannulation procedure was typically completed in 70-80 minutes.

Isolated hearts were instrumented in a similar fashion to the in situ setup; ventricular pressures were measured using Millar catheters placed directly into the RV and LV. The first derivative of LV pressures (dP/dt) was calculated in real time, with maximum (dP/dt_{max}) and minimum (dP/dt_{min}) values reported by the data acquisition software. Flow probes were placed at the aortic and pulmonary vein cannulae (Model 8N, Transonic Systems Inc., Ithaca, NY, USA). The filling pressures of both the right and left sides were held constant throughout each experiment. Furthermore, the resistance to ventricular ejection was held constant, therefore providing a constant afterload by attempting to match it to the *in vivo* aortic/systemic resistance.

Following instrumentation, defibrillation patches were placed on the anterior and posterior portion of the ventricles, and oxygenated Krebs perfusate was supplied to the cardiac chambers. The heart was defibrillated and the experimental protocol initiated. In vitro hearts were divided into three treatment groups: a) control (n=14), b) IL (n=10), or c) DHA (n=16). Figure 7.1 shows the experimental protocol for lipid administration. In each case, the particular compound was circulated for an initial 20-minute period, followed by a 30-minute "working" period during which no further lipids were added. During the subsequent 10 minutes of the study, the entire circulating volume of perfusate was replaced 2-3 times in an attempt to remove all lipid compounds.

IL Administration

Intralipid was delivered continuously (Figure 7.1) in an attempt to maintain a constant

circulating level of lipids in the isolated heart apparatus. A syringe pump was connected

directly to the aortic root cardioplegia cannula to minimize compound dilution and to help

ensure administration of IL into the coronary circulation. The infusion was performed at

0.5 ml/min for the 20-minute administration time, resulting in 10 ml total administered

volume, or 2 g of lipids.
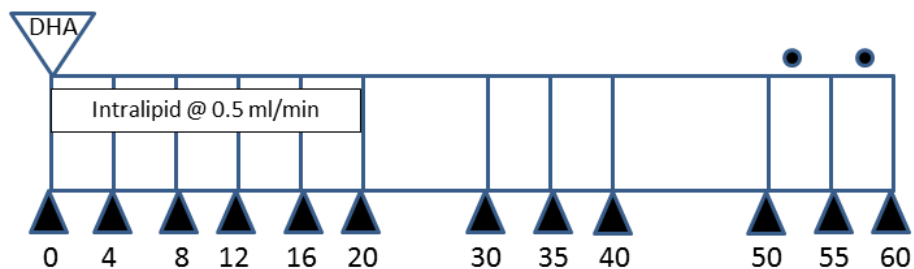
Figure 7.1: treatment timeline. DHA and IL were administered at the times indicated. Hemodynamic data were collected at the times marked by the black triangles. Buffer changes were performed after 50 and 55 minute timepoints, as indicated by the black circles.

DHA Administration

A 0.27 ml bolus of stock DHA (250 mg/ml) was administered at time 0 as shown in

Figure 7.1. This volume delivered 0.0675 g of DHA, with an approximate resultant

concentration of 30 µM. Given the small volume of administered DHA relative to the

circulating volume of Krebs, the injection was made directly into the largest volume

perfusate reservoir of the isolated heart apparatus to help ensure uniform dilution within

the system. This concentration of DHA is similar to that used in other in vitro

experiments [13], [14].

Statistical Analysis

Statistical analysis of the data employed unpaired ANOVA (analysis of variance) along with ttests, employing the bonferroni corrections where appropriate. Comparisons were performed across elapsed time of the experiment, and also between treatment groups. Statistical significance was inferred if $p < 0.05$. Unless indicated otherwise, all values are reported as mean ± standard error.

Results

A total of n=40 swine (mean weight 82.9 ± 9.4 kg) were use in this study. No significant differences existed in cardiac physiology before explant and start of in vitro protocol. Treatment groups were tracked over time as well as compared against each other. In this way, each treatment was compared both against the others and against itself over the course of the in vitro experimentation as time elapsed.

Time Course Comparison

As shown in Table 1, for the maximum left ventricular pressure (maxLVP) did not significantly decrease from baseline with any of the treatments. The minimum LVP (minLVP) increased from baseline only in the DHA, starting at 40 minutes in right sided mode (RtSide) and at 50 minutes in Langendorff mode. The maximum contractility as measured by the time derivative of pressure (dP/dt_{max}) decreased in the control group at 40 and 50 minutes in right sided and Langendorff modes, respectively. The contractility decreased in the DHA treated hearts at both the 50 and 55 minute times, but not at 60 when the hearts were in working mode. These hearts also significantly decreased in right sided and langendorff modes at 12 minutes. The increase in relaxation rates was

measured by the minimum time derivative of pressure (dP/dt$_{min}$). Similar to the

contractility, the control hearts significantly increased relaxation rates in both the right

sided and Langendorff modes, but no in working mode. The DHA treated hearts also

increased their relaxation rates in each of the three modes as seen in Table 1. The IL

treated hearts had no significant changes in any of these four parameters when

compared to baseline.

maxLVP	Control	IL	DHA
work	n/a	n/a	n/a
RtSide	n/a	n/a	n/a
Lang	n/a	n/a	n/a

minLVP	Control	IL	DHA
work	n/a	n/a	n/a
RtSide	n/a	n/a	40
Lang	n/a	n/a	50

dP/dt$_{max}$	Control	IL	DHA
work	n/a	n/a	50,55"
RtSide	40	n/a	12
Lang	50	n/a	12

dP/dt$_{min}$	Control	IL	DHA
work	n/a	n/a	55
RtSide	40	n/a	35
Lang	55	n/a	50

Table 7.1**: Time of observed significant parameter decline. Data resulting from comparisons between in vitro data measured at time 0 (reperfusion) and subsequent time points over the course of an hour. Listed in the table above are the time points after which a significant difference was observed when compared to time 0. A shorter time indicates that performance declined more quickly, while a later time point indicates that performance was better maintained. The case where the difference was observed only at the particular time (as opposed to the indicated time and all times following) is indicated (") Significance was considered where p<0.005 (Bonferroni correction with ten comparisons). MaxLVP = maximum left ventricular pressure; DHA = docosahexanoic acid group; IL = IntraLipid group; work = full-working mode; rtside = right-sided working mode; lang = Langendorff mode; minLVP = minimum left ventricular pressure; dP/dt$_{max}$ = maximum left ventricular pressure time derivative; dP/dt$_{min}$ = minimum left ventricular pressure time derivative.**

Between Group Comparison
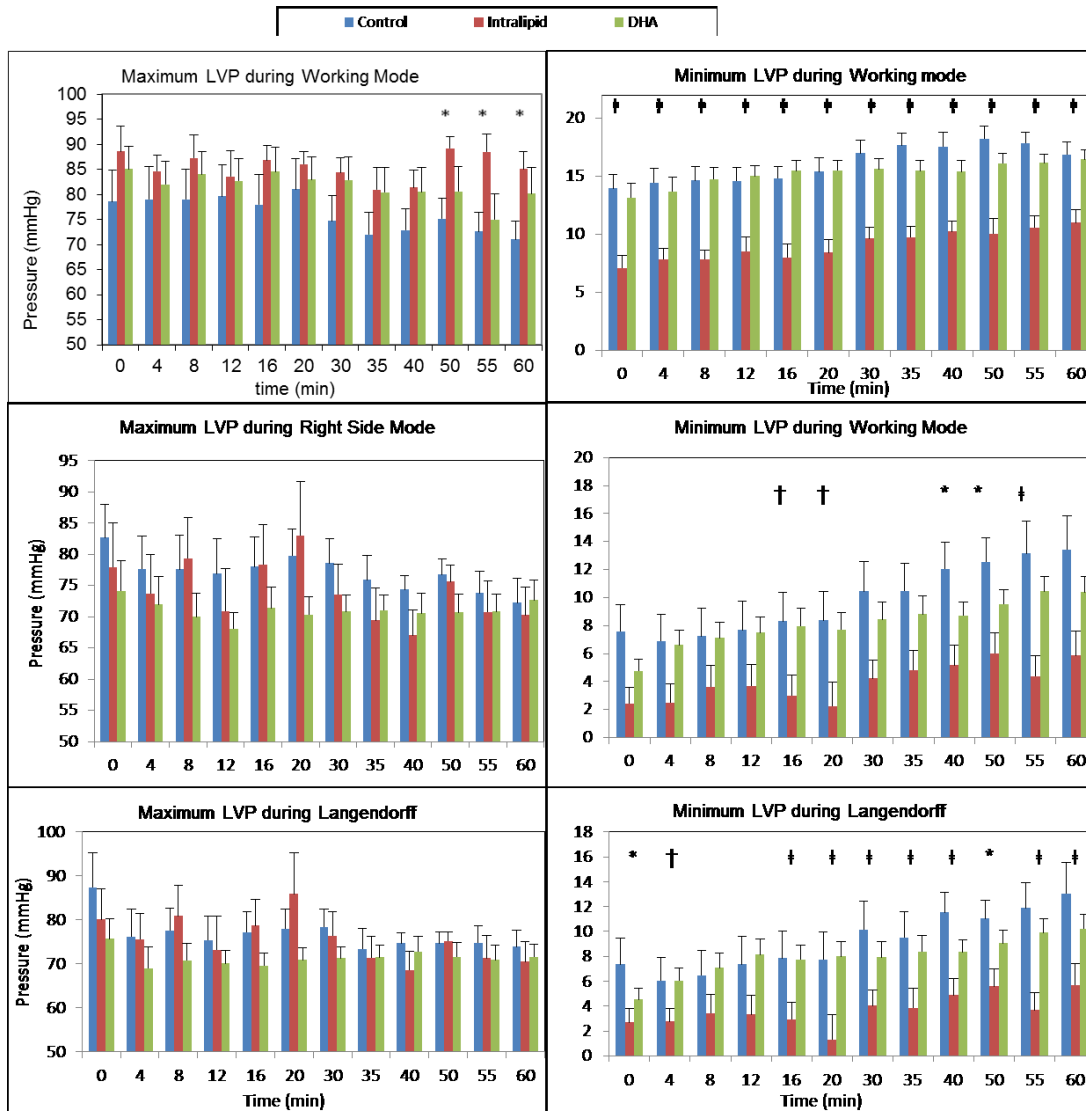
Figure 7.2: Maximum (left) and minimum (right) left ventricular pressures in working mode (top) right side working mode (middle) and Langendorff perfusion mode (bottom). MaxLVP = maximum left ventricular pressure; and minLVP = minimum left ventricular pressure. The * indicates significant difference between IL and control; the † indicates significant differences between IL and DHA; the ‡ indicates significant differences between IL and both DHA and control

Figure 7.2 shows plots of maxLVP and minLVP for each treatment group, as recorded

during A) working mode, B) right sided working mode, and C) Langendorff perfusion

modes. In working mode, IL reached a higher maximum pressure compared control for

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the last ten minutes. The IL also had significantly lower minimum LVP throughout the

hour compared to both control and DHA groups. In the right sided mode, there is little

difference among the maxLVP at each point. The IL treated group had lower minLVP at

most points compared to both control and DHA, marked in Figure 7.2. In Langendorff

mode when the only flow in the heart was the coronary flow, there were no significant

differences in the maxLVP. However, IL treated hearts had significantly lower minLVP at

several points, as marked in Figure 7.2.

Figure 7.3 shows in vitro dP/dt_{max} and dP/dt_{min} values as recorded for the same time

points during the same three perfusion modes. In working mode, IL treated hearts

contracted and relaxed faster than control or DHA hearts, with statistical significance at

the times marked in Figure 7.3. In the right sided working mode The IL hearts had

higher contraction when compared to DHA hearts, with significantly better performance

at 20, 30 and 40 minutes post reanimation, as noted. There were no significant

differences observed in Langendorff mode.
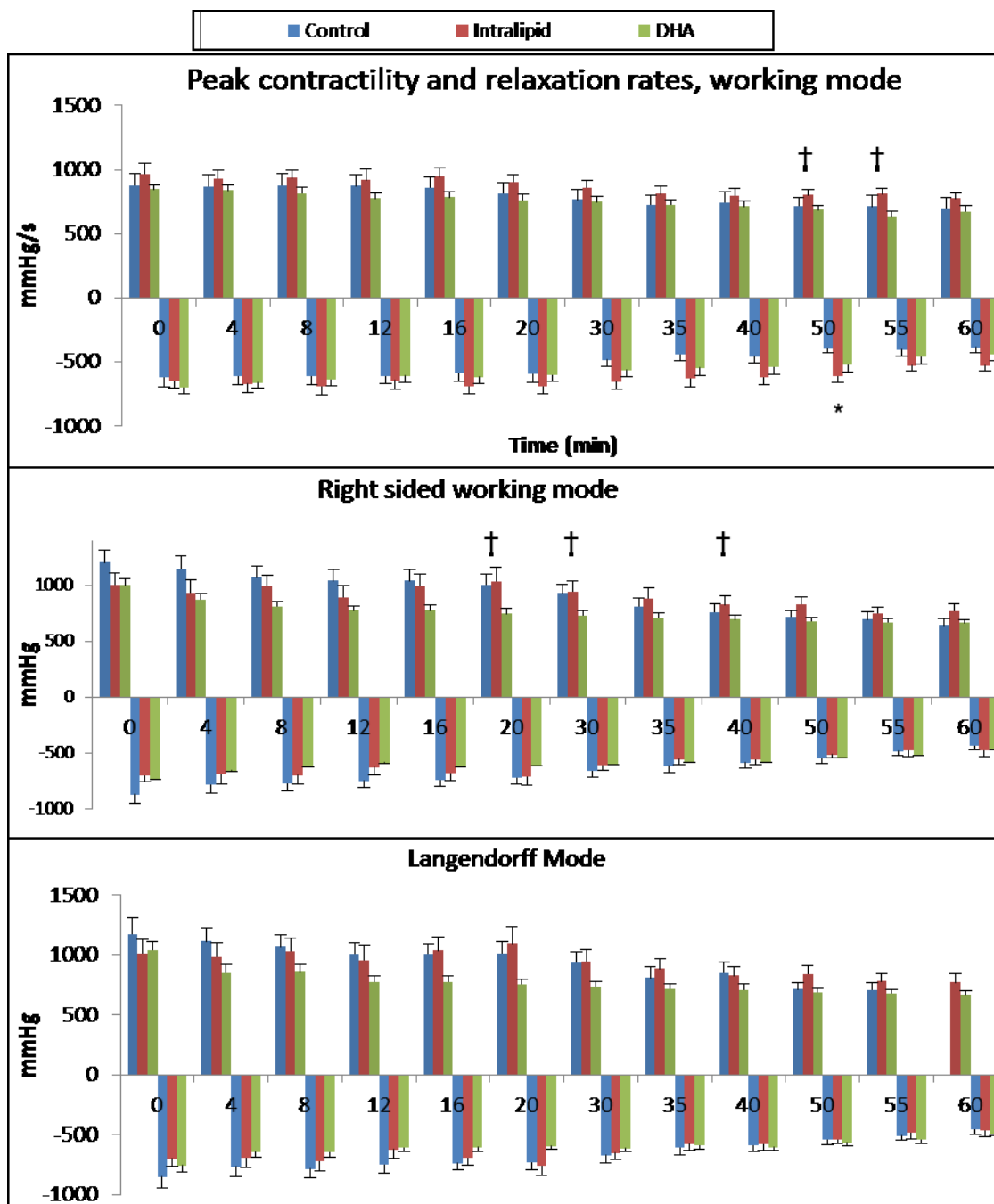
Figure 7.3: Maximum and minimum contractility. The * indicates significant difference between IL and control; the † indicates significant differences between IL and DHA; the ǂ indicates significant differences between IL and both DHA and control

Discussion

Based on previous experimentation, it is assumed that cardiac performance declines over the course of the experiment. While no significant changes were found in maxLVP over time (Table 7.1), this in itself is significant. It should be noted in Figure 7.2 that the control group continued to exhibit a decline in maxLVP as the experiment progressed, while IL actually showed a further increase to values similar to those at time. Overall, administration of lipid compounds was found to better maintain cardiac performance with significant improvements seen in IL treated hearts. In general, IL treatment tended to result in improved cardiac performance over the DHA group. In particular, minLVP was markedly lower over the entire course of the experimentation. In examining the perfusion modes, it can be seen in Figure 7.2 and Figure 7.3 that the most pronounced benefits were observed during work.

The main advantage of performing this study in the isolated heart preparation was the ability to remove systemic influences (neural, hormonal, etc.) and study strictly cardiac effects of the investigational compounds. Full-working mode provides the most accurate basis for comparison given the physiological functioning of the left ventricle. It is in this mode that the Krebs perfusate is circulated in a manner similar to that found in situ. Consequently, the LV is actively filling and ejecting, which allows for better assessment of pressure and contractility measures.

Supplemental Lipids

According to the manufacturer, IL is a phospholipid-stabilized soybean oil compound. Van de Velde and colleagues further identified the major components in this

triglyceridebased lipid emulsion as five different long-chain free fatty acids: linoleic acid (C18:2n6), linolenic acid (C18:3n3), oleic acid (C18:1n9), palmitic acid (C16:0), and stearic acid (C18:0). These compounds (and some others in smaller amounts) are emulsified in 1L of water using 12 g/L of an egg-yolk-derived phospholipid emulsifier consisting mainly of phosphatidylcholine [15]. The recommended dosage when used as a nutritional supplement is approximately 2 g of fat/kg body weight/day.

The healthy "normal" heart can metabolize a wide variety of substrates including fatty acids, glucose, lactates and ketone bodies. Long-chain nonesterified free fatty acids and glucose are often the main energy sources for myocardium. Under normoxic conditions, metabolism is mainly oxidative. Fatty acid metabolism, which occurs predominantly via beta oxidation, is very similar for short-or long-chain fatty acids except that long-chain FAs produce more C2 units in form of acetyl-CoA. The energy production from a molecule of a fatty acid is dependent on the length of its molecular chain. As such, the energy yield from a molecule of a longer-chain fatty acid is higher; however, beta oxidation of unsaturated fatty acids requires special enzymes. Intralipid is comprised of several saturated and unsaturated fatty acids all with shorter chain-lengths than that of docosahexanoic acid (DHA, 22C:6n-3).

Limitations

DHA was supplied in concentrated form, unbound to any carrier molecules. It has been shown that DHA uptake is enhanced in the presence of albumin or another similar molecule. It is not known how the addition of an albumin substrate would have affected the results of the current study.

Translating the proper dosage of IL was unclear given that it is clinically administered into a patient's systemic circulation. It is possible that a lesser amount would have similar functional effects. The choice of delivering 10 ml of IL was made based on work previously published using the same compound to treat isolated hearts [15].

The isolated heart preparation subjects the heart to mild global ischemia. The amount of dissolved oxygen supplied by the Krebs perfusate is considered to be inadequate to support full aerobic metabolism. Consequently, and in conjunction with other factors, hemodynamic performance is known to degrade over the duration of the prep. Further, we were unable to study long-term (> 1 hr) effects due to the necessity of fully utilizing the isolated preparation for other experiments. A wide range of other investigations of was generally carried out following the current study, making it difficult to obtain consistent hemodynamic data from the isolated heart preparation late in the day.

Conclusions

Hemodynamic performance tended to be better preserved with both DHA and IL treatment when compared to control hearts. Overall, IL tended to result in greater improvement in cardiac function; IL increased diastolic performance to a greater degree than systolic, and also to a greater degree than DHA. Given the difference in molecular chain length between DHA and the components of IL, it is unlikely that the benefits of IL administration were due to metabolic reasons. We postulate that the observed hemodynamic benefits were due to integration of lipid compounds into the cellular membrane, thereby modifying the fluidity of the phospholipid bilayer.

Chapter 8: Multifunction Pericardial Drainage Catheter

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Preface

As part of the design of the previous animal experiments, great consideration was given to the clinical application of the therapies. With the help of Eric Richardson, I designed a delivery catheter for use during and after cardiac procedures. This idea stemmed from methods of delivery and access, which was trivial with an open chest procedure, but eliminated upon closure. Chronic delivery with an implanted pump seems possible, but has shown problems in previous studies. In swine, after a soft catheter was left in for six months, the myocardium showed moderate inflammation and scarring [1]. Thus the idea for acute delivery was more suitable. The prototypes we developed are designed to be placed in the pericardial space upon closure after cardiac surgery along with chest drainage tubes. The different interactions of this catheter lead up to a model with multiple lumens for clearing pericardial effusions as well as delivering drugs. An incorporated temporary pacing lead allowed for increased control of heart rate. This work is summarized in *Chapter 8: Multifunction pericardial drainage catheter*, which was presented at the Design of Medical Devices conference in 2011.

Summary

A longstanding controversy in the field of cardiac surgery is whether or not to repair the pericardium on completion of open heart surgery. The device proposed is a multi-lumen catheter that will allow for delivery and removal of fluids to and from the pericardial space. In more detail, the main lumen will allow for gentle suction. A second lumen will allow for targeted drug delivery if desired. Targeted drug deliver can be especially important in the case of adverse events such as infection or postoperative atrial fibrillation. A temporary pacing lead through the catheter will allow for increased rate control by means of anti-bradicardic pacing. The catheter can help reduce the complications involved with closing the pericardium, and thus encouraging more surgeons to complete this task in more procedures in order to reduce complications among the ever-increasing number of repeat operations.

Introduction

The pericardium provides a controlled environment for the heart. The seemingly double-layered membrane is actually one single layer of tissue folded on itself. The inner layer, or serous pericardium, is fused to the epicardium, but the tough outer layer, or parietal or fibrous pericardium, can be almost 0.5mm thick surrounding the heart. The outer layer is the most visible and by itself is often referred to as the pericardium. Between the layers is 15-25mL of pericardial fluid that lubricates the heart as it beats within the mediastinum.

Open heart surgery, which requires the surgical opening of the pericardium, carries inherent risks including infection. Because the pericardium is a contained space, any buildup of fluid that may accompany an infection will cause cardiac tamponade; a condition in which the heart is prevented from filling effectively as a result of the fluid buildup in the pericardium. In order to avoid this risk, many cardiac surgeons will leave the sac open rather than attempt to close it. Leaving the pericardium open lowers the risk of cardiac tamponade, but can lead to other issues. Without the pericardial barrier separating the two, scarring between the myocardium and sternum can occur, effectively fusing the ventricles to inside of the sternum. In other words, the pericardium that remains open will often not provide the same protection as the pericardium that is sutured together. This barrier is especially important since an increasing number of patients are undergoing a second or third cardiac procedure, and separating the heart from the sternum carries significant risks such as damage to myocardium itself and increases the overall surgical time. In 2001 it was estimated that 5% of all cardiac surgeries in the UK were redo operations, the second or third on a single patient [1].

Currently, most cardiac surgeons do not attempt to close the pericardium because they feel that the benefit of removing the pericardium outweighs the drawbacks. However, a minority of surgeons still argue that the benefits closing the pericardium warrant closure. Clearly either approach has disadvantages and benefits to the patient; the device proposed here removes and reduces some of the risks incorporated with pericardial closure thus encouraging more frequent closure.

Another complication with cardiac surgery is the high rate of arrhythmias such as atrial fibrillation, which occurs post-operatively: such occurrence has been reported to be as high as 18-34% in post-surgical patients, depending on the procedure [2]. As a result, it has become common to administer prophylactic antiarrhythmic drugs [3], but this also does not come without risks. While preemptive treatments have proven effective [4], most antiarrhythmic agents also have inherent side effects that make prophylactic treatment not suitable for all patients. Intrapericardial delivery of antiarrhythmics has been shown to produce similar or greater tissue concentrations compared to intravenous delivery [5, 6], while reducing drug levels throughout the rest of the body. Such an increase in tissue concentration achieved through pericardial delivery should have similar effects to delivery by other means.
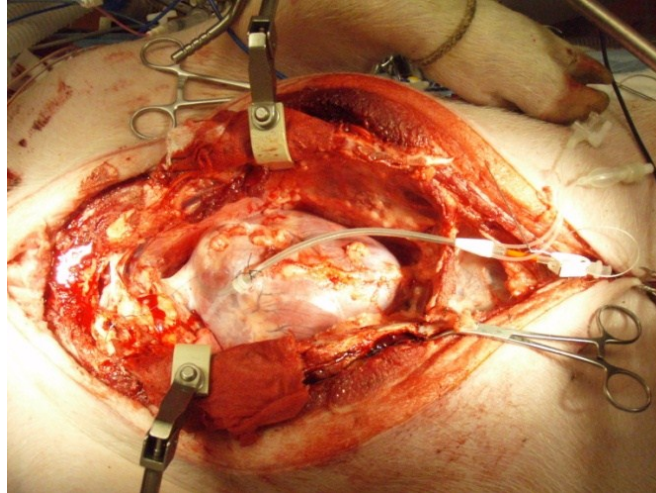
Figure 8.1: The pericardial drainage catheter inserted into the pericardium of a pig. Here a suture around the shaft holds the catheter in place. Other options to hold the catheter in place include inflating a balloon to hold it in place.

Device Description

The device proposed (Figures 8.1 and 8.2) can either incorporate double or triple lumen catheter that will allow for delivery and removal of fluids to and from the pericardial space. In more detail, the main lumen will allow for gentle suction. Because of this vacuum, the catheter must remain rigid enough to prevent collapse while at the same time remaining soft enough so as not to irritate the epicardial surface of the heart. The catheter shaft should employ a higher durometer (or could be reinforced with a mesh) while the tip and portion inserted into the pericardium (and against the heart) would best to be a softer material such as silicone. The distal tip should also have multiple openings to reduce the chance of clogging while suction is applied. From early prototype design feedback, we approximate that the main lumen should be approximately 9 Fr (3mm) in diameter. This would be of sufficient size to clear typical amounts of pericardial effusion in such surgical cases.

A second incorporated lumen will allow for targeted drug delivery as desired. Such targeted drug deliver could be especially useful in the surgical cases of adverse events such as infection or postoperative atrial fibrillation. As such, the second lumen would provide for bolus or continuous delivery of antibiotics or antiarrhythmic agents. In those cases in which the pericardium was repaired, the delivered drug will remain within the contained space and decrease systemic side-effects of the selected drug.

In order to keep the catheter in place post-surgically and also allow for enhanced removal, an optional third lumen could be connected to a balloon near the distal tip of the catheter. This would enable the surgeon to cut or suture a small hole, insert the catheter and inflate the balloon just inside the pericardial space. The balloon would anchor the catheter and, when deflated, would allow for easy removal. Another option to prevent the catheter from slipping would be to add a small hook or attach it to a temporary pacing lead.

A temporary pacing lead through the catheter (or attached to the outside) will allow for increased rate control by means of anti-bradicardic pacing. A patient may have irregular heart rhythm after surgery, or may even have a sensitive reaction to certain antiarrhythmics, such as beta blockers, which will result in a slower heart rate. This pacing lead could travel through the skin on or in the catheter, or could remain as a separate entity. The pacing lead could also be sewn into the epicardium, and this may also provide a means to hold the catheter assembly in place.
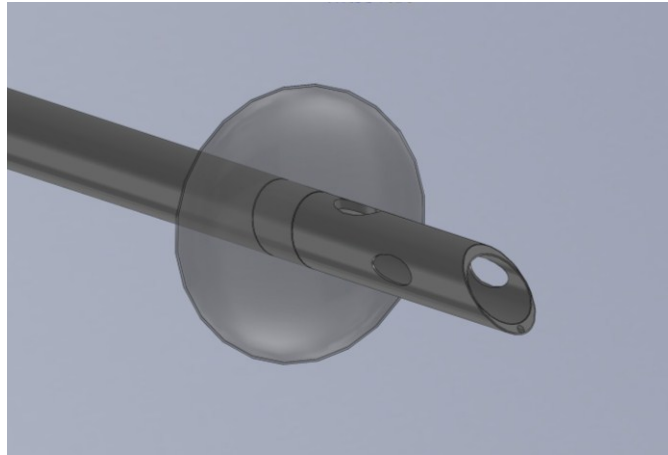
Figure 8. 2: Catheter tip with balloon. Main lumen is used for drainage while smaller lumen (visible at distal tip) is used for drug delivery. The optional balloon shown would help keep the catheter in place.

The delivery/drainage/pacing device designs described here could allow the given cardiac surgeon to close the pericardium while reducing the risk and consequences of infections. The risks of tamponade would be greatly reduced with the drainage function. In the case of infection or arrhythmia, the catheter would also provide a conduit to target antibiotic or antiarrhythmic treatment directly onto the heart. In addition to pharmacological rate control, the accompanying pacing lead allows the addition of pacing, granting greater post-surgical control of heart rate. It is considered that these catheters would remain in place for a short period of time, with the idea to remove at the same time as a chest tube, typically less than 48 hours.

Limitations And Conclusions

One limitation to pericardial closure is that it slightly reduces initial cardiac index and stroke work. Rao et al. found that these functions were reduced one hour

postoperatively in patients who had pericardial closure (*P*<0.001). However, they found

no significant difference in function at 4h or 8h postoperatively [7].

In the cases of severe bleeding into the pericardial space, the catheter may not be large

enough to keep up with blood loss. Yet, in these types of cases, where the patient is

bleeding several hundred mL/hr, additional intervention would be likely warranted.

It should be noted that since the pericardial barrier would remain primarily intact, this

catheter would not be a replacement for, but rather a supplement to, a typical chest tube

placement. The additional transcutaneous tube increases the risk of irritation and

infection, however, current practices minimize these risks.

Overall, the catheter systems proposed here could help reduce the known complications

involved with closing the pericardium. We propose that by making the closure of the

pericardium safer procedure, it would be possible to encourage more clinicians to

complete this task in more surgical cases. In addition to the targeted drug delivery, a

closed pericardium will have the benefit of reducing complications in future surgeries.
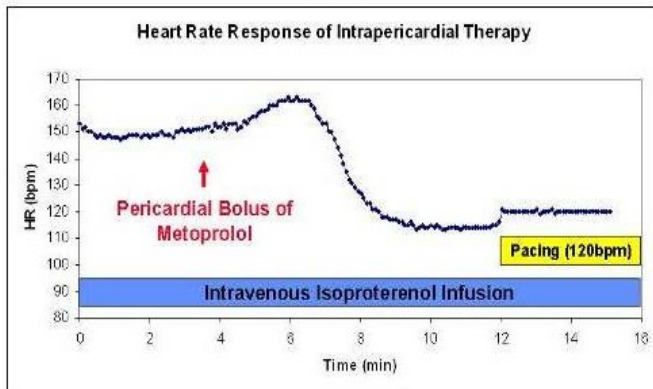
Figure 8.3: Heart rate through a pilot study exhibiting the properties of the catheter, which is placed in the pericardial space in a swine model. At time t=0, the heart rate has been elevated with isoproterenol to induce sinus tachycardia. At the marked time, a bolus of metoprolol is given to the pericardial space. After a slight bump, the heart rate drops and levels off 40bpm lower to 110bpm. To illustrate further control, at minute 12, the heart is paced at 120bpm

Figure 8.4: A version of the drainage catheter that contains a pacing lead and adequate size for withdrawing pericardial effusions. This version also contains a wick (gauze as pictured) that will aid in keeping treatment next to the myocardium and might be necessary in cases where the pericardium is not closed.

Figure 8.5: (below) Poster exhibited at the Design of Medical Devices conference in 2011

Multifunction Pericardial Drainage Catheter

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The **VISIBLE HEART**® Laboratory
Lab I eatures International Systems Physiology

Abstract

A longstanding controversy in the field of cardiac surgery is whether or not to repair the pericardium on completion of open heart surgery. The device proposed is a multi-lumen catheter that will allow for delivery and removal of fluids to and from the pericardial space. In more detail, the main lumen will allow for gentle suction. A second lumen will allow for targeted drug delivery if desired. Targeted drug delivery can be especially important in the case of adverse events such as infection or postoperative atrial fibrillation. A temporary pacing lead through the catheter will allow for increased rate control by means of pacing if the heart becomes bradycardic. The catheter can help reduce the complications involved with closing the pericardium, and thus encouraging more surgeons to complete this task in more procedures in order to reduce complications among the ever-increasing number of repeat operations.

The Pericardium

- Provides controlled environment for the heart
- Made up of two layers:
  - Serous pericardium is fused to the epicardium
  - Parietal (fibrous) pericardium is tough, thick layer that is often referred to as the pericardium
- Between the layers are 15-25mL of pericardial fluid that provide lubrication and cushion for the heart

Background

- Cardiac surgery has inherent risks, such as infection and postoperative atrial fibrillation
- Leaving the pericardium open can often lead to scarring between the sternum and heart
- Reoperation becomes difficult due to scarring
- Closing the pericardium can provide a barrier to prevent such scarring
- Closing the pericardium also carries risk, such as tamponade

Procedure Outline

During surgical cardiac procedures, whether open chest or minimally invasive, the pericardium is opened. Upon completion of the surgery, most often the pericardium is left open. The proposed device will allow the surgeon to close the pericardium by minimizing the risks involved. With the device, additional benefits will also be seen, including optional targeted drug delivery.

- Open chest via thoracotomy or sternotomy
- Open pericardium to access the heart
- Complete intervention. Close heart
- Close chest and chest drain placed
- Tube(s) removed within 48 hours unless complications arise

- Close pericardium, placing pericardial drainage catheter
  - Proposed catheter placement

Major Risk Summary

SURGERY ALONE	WITH CLOSING PERICARDIUM	WITH MULTI-LUMEN CATHETER
- Infection	- Infection	- Infection
- Atrial fibrillation	- Atrial fibrillation	- Atrial fibrillation
- Scar formation	- Scar formation	- Scar formation
	- Tamponade	- Tamponade

Device

DESCRIPTION:
- Multi-lumen catheter
  - Main lumen diameter is approximately 3 mm
  - Second lumen allows for targeted drug delivery
  - Optional third lumen could be used to inflate a balloon to hold catheter in place
- Incorporated with a temporary pacing lead for increased rate control

USE:
- Apply gentle suction to remove excess fluid from pericardial space
- At first sign of infection, give dose of antibiotics IV and/or to pericardium
  - If arrhythmias such as atrial fibrillation develop, deliver antiarrhythmic drugs directly to pericardial space
 - If patient becomes bradycardic, start pacing

Limitations

- One limitation to pericardial closure is that it slightly reduces initial cardiac index and stroke work, but only in the short term.
- The catheter may become clogged or overrun by sever bleeding.
- This catheter would not be a replacement for, but rather a supplement to, a typical chest tube placement.

Conclusions

Overall, the catheter system proposed here could help reduce the known complications involved with closing the pericardium. We propose that by making the closure of the pericardium a safer procedure, it would be possible to encourage more clinicians to complete this task in more surgical cases. In addition to the targeted drug delivery, a closed pericardium will have the benefit of reducing complications in future surgeries.

Chapter 9: A device and methodology for continuous hypothermic perfusion of explanted large mammalian hearts, followed by in-vitro langendorff reanimation: pilot studies

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Preface

In addition to the pre- and post-treatments to improve function of the heart, the cold ischemic time during a typical heart transplant provides another opportunity for cardioprotective treatments. To investigate treatment during this time, I helped Robin Brusen, an MD/MS student, design and test a continuous perfusion cold storage apparatus, which is described in *Chapter 9: A device and methodology for continuous hypothermic perfusion of explanted large mammalian hearts, followed by in-vitro langendorff reanimation: pilot studies*, published in the Journal of Medical Devices. In the pilot studies, several additives were included in the cold perfusate: albumin, DHA, DADLE (a δ-opiod agonist), lidocaine as well as a mid-thermic interval. In these pilot studies, the hearts were ischemic for 15-24 hours, and while hemodynamic function was below normal levels, the results were superior to those from static cold storage.

Summary

The current methodologies of clinical heart transplantation limit the ischemic window to 4-6 hours. Periods longer than this can induce dysfunction in the organ and lead to increased patient morbidity and mortality. An alternative to the current methods of static cold storage (CS) is continuous hypothermic perfusion (CHP), where a hypothermic oxygenated crystalloid solution is mechanically perfused through the coronary arteries. This has been shown to preserve function for up to 72 hours, but the techniques have yet to be optimized. We have developed an apparatus and methodology for performing CHP on large mammalian hearts, followed by reanimation in our in vitro Langendorff apparatus (Visible Heart™). We are also investigating the utility of the cardioprotective agents docosahexaenoic acid (DHA) and [D-Ala2, D-Leu5] enkephalin (DADLE), both of which have shown cardioprotective effects in our laboratory; we believe that their addition to the preservation solution can further extend the transplant window. A series of pilot studies have been performed to date, with modestly successful results. Hearts preserved with CHP seem to show better functionality than CS hearts, but far worse functionality and higher release of biochemical injury markers than hearts reanimated immediately after explant. We hope to use this system to optimize CHP methodology and eventually develop a system for prolonging the window for heart transplantation.

Introduction

The advent of heart transplantation in 1967 [1] brought hope for millions of patients with late-stage heart failure. The complete exchange of a diseased heart for a healthy one has the potential to prolong life for many years, especially in individuals who otherwise would likely have died in several months or would have required support on a ventricular assist device. Nevertheless, even in best-case scenarios the heart transplant procedure presents complications, difficulties, and an average longevity of 10 years post-transplant.

It is the relative degree of ischemic injury associated with the transit of the heart from donor to recipient that lies at the core of many complications attributed to the heart transplantation procedure. In general, all transplanted organs/tissues undergo some amount of ischemic damage, but cardiac tissue seems to be particularly susceptible. More specifically, the kidney and liver can be transplanted reliably and safely for a cold ischemic time of up to 24 hours, whereas for the heart this period is currently clinically limited to 4-6 hours. Importantly, ischemic times beyond these considered limits have been shown to increase the relative risks of recipient mortalities at 1 year [2].

The current standard of heart preservation for transplantation is static cold storage (CS) where the donor heart is arrested with a cardioplegic solution and placed in a simple ice-filled cooler. This is primarily based on the principle that metabolic activity will be suppressed by hypothermia, thus reducing enzymatic rates. The generally accepted rule is that metabolic rate is reduced by a factor of 2 for every 10°C decrease in temperature, yielding a remaining 10-12% of basal metabolic rate at 4°C. It is important to note that this residual metabolic activity continues during the CS transportation and, in the

absence of oxygen and a supplied energy source, leads to anaerobic metabolism and gradual decreases in both tissue ATP levels and pH. Ultimately this sustained metabolism leads to ischemic myocardial damage and primary graft dysfunction [3]. This clinically translates to decreased cardiac contractility after transplant, typically requiring additional inotropic and/or mechanical support; the current estimate is that this will occur in 10-25% of recipients even with the current standards concerning donor quality and ischemic times [4]. It should be noted that previous studies in our lab, in which we attempted 17 hours CS preservation followed by in vitro reanimation, yielded minimal or no contractile and/or sustained electrical functionality [5].

One potential method of prolonging the window of heart viability and reducing ischemic damage is continuous hypothermic perfusion (CHP). This procedure is relatively simple and involves continuous perfusion of the coronary arteries with a hypothermic oxygenated crystalloid preservation solution to support residual metabolic activity and thus limit global ischemic damage. More specifically, in one study the use of this technique was shown to shift metabolic activity from anaerobic to aerobic and maintain cell integrity and functionality [6]. CHP as a research method was first reported for application in dog hearts, only a year after the first successful human heart transplant. Importantly in this study, these hearts were shown to maintain contractility for up to 72 hours [7]. Subsequently, this approach was employed to preserve both baboon and swine hearts for up to 48 hours before implantation into recipients [8]. Furthermore in an additional report, CHP was shown to be superior to CS with 6-hour preservation times; CHP resulted in better function upon reanimation, preserved high tissue ATP concentrations, and lowered levels of several markers for oxidative damage, apoptosis,

vascular dysfunction, and DNA damage [9]. Taken together, these results suggested that not only could CHP lengthen the transplant window, but it could also decrease primary graft dysfunction associated with short ischemic times.

To date, CHP has been relatively well received by the kidney transplantation community, and several commercial devices for renal transplantation are currently available [10]. Interestingly, CHP for clinical heart transplantation has yet to be adapted, despite the fact that it has been shown advantageous over CS preservation in animal models. On the other hand, one can identify several issues with using CHP for extending the window of heart transplantation, the main disadvantage being its potential for the induction of myocardial edema and associated diastolic dysfunction. In previous studies, this edema has been reported to increase the weight of hearts up to 5-fold compared to those recovered with CS for the same time interval, despite using preservation solutions with high oncotic pressure [11]. This, combined with low numbers of clinical trials, a lack of consensus on methodology in preclinical experiments, and long length and high cost of the experiments has led to a lack of clinical acceptance. Thus more research must be performed on such techniques to perfect or enhance them before they will become a clinical reality.

It is proposed by our laboratory that improved formulations of the preservation solution could lead to better function of hearts preserved with CHP. More specifically, we postulate that the addition of docosahexaenoic acid (DHA), an ω-3 fatty acid and/or [D-Ala2, D-Leu5] enkephalin (DADLE), a δ-opioid agonist could be employed to further limit ischemic damage to the heart. For example, there are numerous known actions of DHA,

including increased fluidity of cell membranes, ion channel blockade, and inhibition of eicosanoid pathways [12]. Furthermore, our laboratory has previously shown that intrapericardial administration of DHA followed by coronary artery occlusion can reduce associated infarct sizes and incidences of ventricular tachycardia relative to controls [13]. It should also be noted that δ-opioid agonists have been shown to induce the hibernating state in mammalian tissue, leading to more stable membrane potentials, stabilized ion gradients, altered channel activity, decreased arrhythmia propensity at low temperatures, reduced vascular dysfunction, and/or inhibition of oxidative damage [14]. As such, we hypothesize here that both of these compounds can be used to reduce ischemic damage when combined with the CHP methodology.

In this paper, we describe our novel device for performing CHP on swine hearts, the *in vitro* apparatus for reanimation, a series of pilot studies to assess currently reported CHP methodology, and plans to develop it further.

Methods

Swine (70-100 kg) were used for such investigations following approval from the University of Minnesota's Animal Care and Use Committee. Intramuscular telazol (5 mg/kg) was used to preanesthetize the animals, followed by intravenous administration of thiopental (5 mg/kg) via an ear vein. The animals were intubated with a 7- or 7.5-mm inner diameter endotracheal tube and mechanically ventilated with 65% air and 35% O2. Subsequently, general anesthesia was maintained using isoflurane (~1.5 minimal alveolar concentration); end-tidal CO2 and exhaled isoflurane levels were monitored continuously and adjusted to maintain sufficient anesthetic depth. The animals' hearts

were then exposed via median sternotomy. In several of the pilot studies, incisions into

the pericardial sac were made and then a soft infusion catheter was inserted to allow for

the continuous infusion of DHA (45 mg dissolved in 20 mL saline solution, infused over

45 minutes). After heart exposure and/or DHA pretreatment, cardioplegia cannulae were

sewn into the aortas and St. Thomas II Cardioplegia solution (1 L) was infused into the

cannulae with a perfusion pressure of 150 mm Hg. After contractile and electrical

activities ceased, the hearts were excised, the great vessels were dissected, and plastic

cannulae were tied into the aorta proximal to the innominate artery and distal to the

coronary artery ostia. The heart chambers were flushed with St. Thomas II Cardioplegia

solution (~1 L) to remove residual blood and the hearts were weighed. The hearts were

then connected to the perfusion apparatus and suspended within the lower reservoir.

The perfusion apparatus is shown in Figure 9.1. A circulator-chiller (RM6, Lauda-

Brinkmann, Lauda- Konigshofen, Germany) was used as the pump, refrigeration unit,

and reservoir for the preserved hearts. This device has proven efficacious and safe for

supporting muscle tissue in our laboratory. The fluid was pumped to an upper reservoir

to prevent air bubbles from entering the grafts. Auxiliary tubing connecting the upper to

the lower reservoir prevented overflow. The upper reservoir drained into a 27 μm blood

filter (Affinity, Medtronic, Inc., Minneapolis, MN, USA) and a hollow fiber oxygenator

(Affinity, Medtronic, Inc. Minneapolis, MN, USA) before draining into the cannulated

aortas of the hearts suspended in the lower reservoir within the circulator-chiller.

Surgical tubing (Nalgene Labware, Rochester, NY, USA) was used to connect the

elements of the system. The height of the upper chamber and screw clamps (Nalgene

Labware, Rochester, NY, USA) were used to control the perfusion pressures. A Luer

taper connector between the oxygenator and the heart was used to monitor pressure (Uniflow, Baxter Healthcare, Irvine, CA, USA) and oxygenation (ISO2, World Precision Instruments, Sarasota, FL, USA) of the perfusate.

The durations of the preservation were variable between the preliminary studies described here. The temperatures of the perfusate were either held at a constant 9°C for the duration of the preservation interval or elevated to 25°C for the first 30 minutes of the preservation, then lowered to 9°C for the remainder of the preservation (midthermic interval). The perfusion pressures were set to 15-20 mm Hg at the beginning of the study and no attempt was made to modify them afterwards. Carbogen (95% O_2/5% CO_2) was infused into the oxygenator at a rate of 1 L/min. Samples of the preservation solutions were taken at approximately 3, 6, and 12 hours into the preservation intervals and stored for later biochemical analyses.
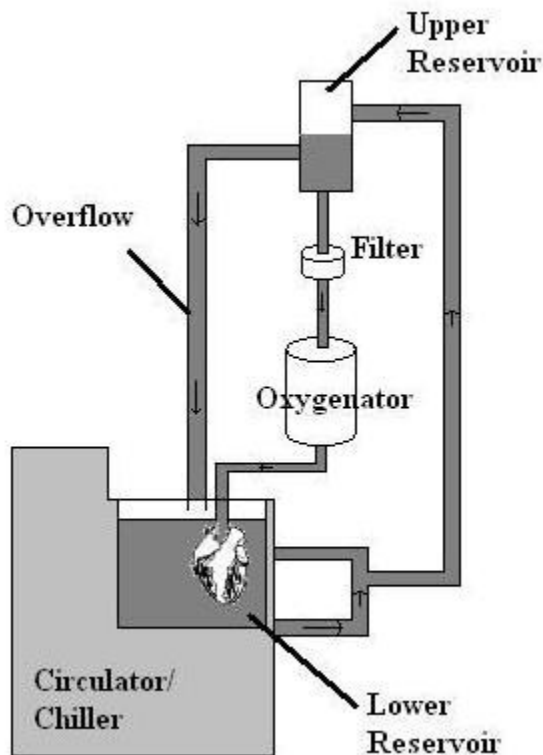
Figure 9.1: diagram of preservation apparatus, showing the circulator/chiller pumping fluid into the upper reservoir, filter, oxygenator, and eventually into the aorta of the preserved heart in the lower reservoir

To date, it should be noted that there is no consensus regarding the best organ

preservation solution to use for CHP. For the present studies, we employed 6 L of

modified Celsior solution. The solution modifications included: 1) addition of albumin

toincrease oncotic pressure and reduce tissue edema; 2) reduction in potassium

concentrations or addition of lidocaine to reduce vascular dysfunction during

preservation; and 3) addition of DHA or DADLE as preconditioning protective agents.

At the end of each preservation interval, each heart was reanimated using the in vitro

Langendorff (Visible Heart™) methodologies which employ a modified Krebs-Henseleit

Buffer (KHB, ~8 L) for approximately 1 hour. The KHB was supplemented with nitroprusside (40 mg) before reanimation to induce vasodilation of the already constricted vasculature as well as dobutamine (~ 0.2 mg/l) to improve contractility. This apparatus and methodology have been previously validated as a method to reanimate large mammalian hearts and assess physiology and functionality [15]. To date, our lab has reanimated and assessed the function of over 700 swine and 39 human hearts using this approach, thus this technique was used in this experimental model as a means for appropriately assessing viability of these potentially preserved hearts. Additionally, ventricular pressures (MDE Escort, Viasys Healthcare Technology, Conshohocken, PA, USA) and surface EKG (101 Patient Monitor, IVY Biomedical Systems, Branford, CT, USA) were monitored during reanimation. Further, for the final two pilot studies, an infusion catheter was placed in the coronary sinus for sampling of the KHB at 0, 20, and 40 minutes after reanimation for biochemical analyses; note that similar samples were drawn from hearts reanimated immediately after explant (within 1 hour) for comparisons.

The preservation solutions and KHB samples were stored at -4°C. After all experiments were completed, the samples were thawed by allowing them to return to room temperature, and 7 mL aliquots of each sample were sent to the Department of Laboratory Medicine for myoglobin and troponin quantification (via radioimmunoassay). Oxidative stress was quantified using a fluorometric thiobarbituic acid reactive substance (TBARS) assay (Cayman Chemical Company, Ann Arbor, MI, USA). Note that malondialdehyde (MDA) is a product of lipid peroxidation that reacts with thiobarbituic

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acid (TBA) to form an adduct that can be measured colorimetrically at 530-540 nm, and the degree of absorbance correlates to the degree of oxidative damage [16].

The data were plotted using Microsoft Excel. Statistical analyses (inference value calculations) were performed using the statistical package R (http://www.rproject.org). Biochemical values were reported as mean ±SE and inferences were performed using Student's Ttests. Regression line parameters were reported as mean ±SE, and P-values were based on the 2-sided alternative model, i.e., slope ≠ 0. Regression models were compared using ANCOVA (F- and P-values reported). Values are reported as significant if $P < 0.05$.

Results

To date, we have performed numerous control and pilot studies employing the system described. Table 9.1 summarizes these preliminary data.

The carbogen delivery rate of 1 L/min saturated the perfusate oxygenation to 95% of atmospheric pressure. Given the small number of studies performed to date and that none had identical conditions, no specific conclusions can be drawn about the relative differences identified in our studies. It is noted, however, that these hearts developed far lower contractility and pressure than those reanimated within one hour after explant, yet they seemed to develop better contractility and pressure compared to hearts reanimated after 17 hours of CS preservation. It was observed that the addition of albumin to the perfusate did not seem to reduce edema, and the degree of edema appeared to be related more strongly to the preservation duration. Interestingly, the addition of DHA

seemed to improve contractility relative to the control studies. Also noted, the pilot study

with the lidocaine additive seemed to be more susceptible to ventricular fibrillation upon

reanimation. Nevertheless, these statements need be considered as anecdotal and merit

further controlled studies to draw definitive conclusions. However we can be reasonably

certain that hearts preserved via the methods described have significantly diminished

function compared to control hearts (~1 hour ischemic time).

The KHB myoglobin and MDA concentrations in the preserved and immediately

reanimated (nonpreserved) hearts are compared in Figure 9.2. There was significantly

higher myoglobin (P = 0.002) and troponin (P = 0.022) released by the preserved hearts

compared to non-preserved hearts, but there were no significant differences in MDA

concentrations (P = 0.111) between the 2 groups.
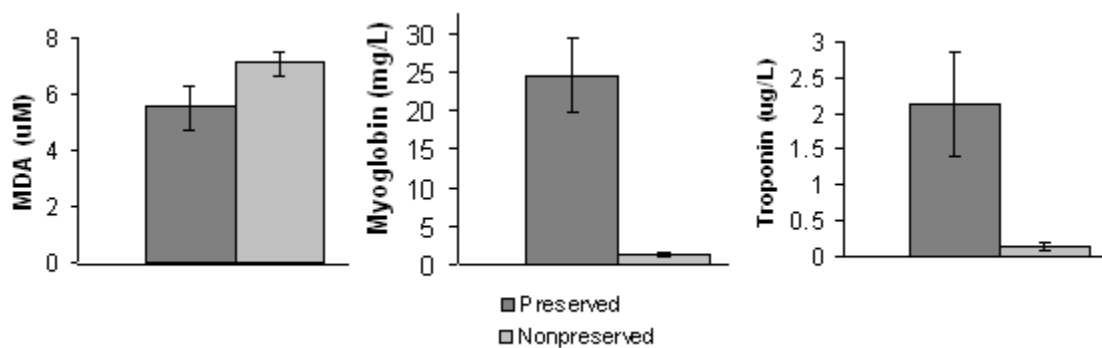
Figure 9.2: comparison of biomarkers in the khb from preserved and nonpreserved hearts, displayed as mean ± se. Student's ttest: mda p-value = 0.111; myoglobin p-value = 0.002; troponin p-value = 0.022.

Of interest, troponin was generally undetectable in the preservation solution. The MDA

concentrations of the preservation solution are shown in Figure 9.3 (A); note that a linear

relationship was assumed between MDA concentration and time. The data were separated into studies with and without the DHA additive. The residuals of each regression model were approximately normal with means centered at 0 (not shown). For the studies with the DHA additive in the preservation solution, the regression line had a slope of 0.025 ± 0.063 (P = 0.695) and R^2 = 0.023. For the studies without the DHA additive, the regression line had a slope of 0.979 ± 0.512 (P = 0.152) and R^2 = 0.550. The ANCOVA comparing the 2 regression lines yielded F = 0.165, P = 0.696.

The myoglobin concentrations of the preservation solutions are shown in Figure 9.3 (B). A linear relationship was assumed between myoglobin concentration and time. The data were separated into studies with and without the DHA additive. The residuals of each regression model were approximately normal with means centered at 0 (not shown). For the studies with the DHA additive to the preservation solution, the regression line had a slope of 38.43 ± 17.23 (P = 0.06) and R^2 = 0.41. For the studies without the DHA additive, the regression line had a slope of 260.56 ± 58.75 (P = 0.02) and R^2 = 0.87. The ANCOVA comparing the 2 regression lines yielded F = 4.974, P = 0.06.
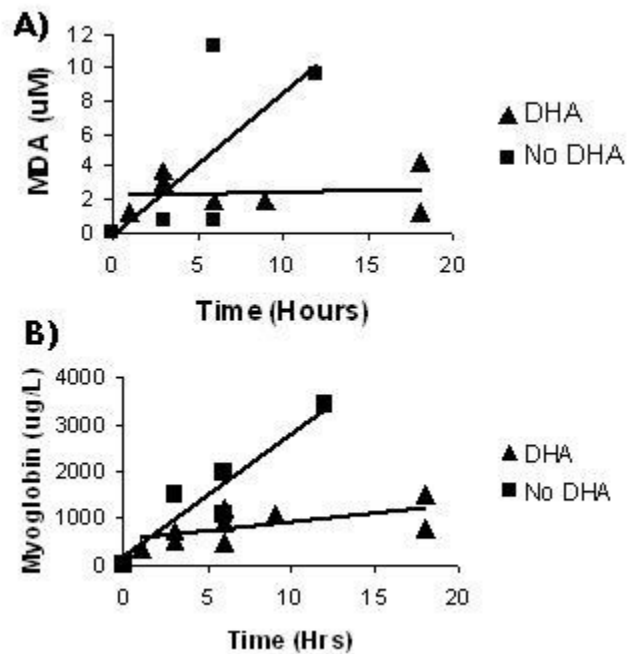
Figure 9.3: mda (a) and myoglobin (b) concentrations of the preservation solution.

Discussion

The pilot studies performed and described here show promise as: 1) an experimental means to assess the outcomes relative to optimizing CHP or the heart for long-term storage; and 2) providing novel pharmacological approaches to precondition the heart to enhance the potential benefits of CHP. Nevertheless, many more studies are needed to validate and build on these aforementioned statements.

Within the pilot studies described here, in all cases we saturated the preservation solution with oxygen (~95%), which is higher than what would be required to maintain the organ at 10°C assuming 100% oxygen extraction. This was done because it remains unknown as to exactly what the oxygen extraction capabilities of the organ are in the

hypothermic arrested state. Nevertheless, given the inhibition of enzyme and hemoglobin function, it was assumed they would be compromised. It is believed that higher oxygen concentrations would be required to support aerobic metabolic activity than if the organ had the same oxygen consumption at normothermia. The oxidative effects of oxygen should also be determined, as the preserved organs are much more susceptible to oxidative damage in the absence of functional antioxidant enzymes. One should note that a higher than necessary oxygen concentration could thus be detrimental to the organ via exacerbating oxidative damage. Furthermore, no studies to date have included glucose or insulin, although previous studies suggest that glucose utilization is minimal during CHP [17]. As such, the effects of various levels of oxygenation should be explored further, perhaps by looking for markers of oxidative damage at varying levels of oxygen concentration.

Study #	1	2	3	4
Preservation Solution	5 L Celsior w/ 0.5% wt Albumin	5 L Celsior w/ 0.5% wt Albumin, 29.3 µM DHA	5 L Celsior w/ 0.5 wt Albumin, 29.3 µM DHA, 2 µM DADLE	5 L Celsior with reduced KCl (5 mM) added Lidocaine HCL (1 mM), titrated NaOH to adjust pH to 7.3
Preservation Duration (hours)	22	24	18	15
DHA Pretreatment	Y	Y	Y	N
Midthermic Interval	N	N	Y	Y
Post-preservation Weight Increase (%)	85.2	78.1	81.8	67.5
Post-preservation Sinus Atrial Rhythm	Positive	Positive	Positive	Positive after multiple defibrillations and administration of dobutamine, calcium
Post-preservation Sinus Ventricular Rhythm	Positive	Positive	Positive	Positive after multiple defibrillations and administration of dobutamine, calcium
Post-preservation Atrial Contractility	Positive but minimal for first 15 minutes of reanimation, then absent	Positive but minimal	Positive but minimal	Positive but minimal
Post-preservation Ventricular Contractility	Absent	Positive but minimal	Positive but minimal	Positive but minimal

Table 9.1: Summary of pilot study results

It should be noted that our Visible Heart™ methodology used for reanimation does not perfectly simulate the functional and metabolic state of a transplanted heart. Importantly, the KHB circulated throughout our apparatus does not contain an oxygen carrier, thus the reanimated hearts continue to be globally ischemic. This contrasts to transplanted hearts, where the high O2 content of the blood allows for better metabolic support of the already ischemic organ. Thus, we would expect metabolically stressed hearts reanimated in our system to perform less well than a transplanted heart.

Biochemical comparisons of the KHB collected from the coronary sinus of preserved and control hearts suggest significantly higher myolysis in the preserved hearts than controls, as evidenced by the myoglobin and troponin concentrations. These proteins are commonly used biomarkers for cardiac injury clinically, and are typically part of the diagnostic criteria for identifying acute myocardial infarction. Since the myoglobin and troponin seemed to correlate well to the perceived function of the reanimated heart, it suggests that these markers could also be used to assess general organ viability. Further, these biomarkers have a distinct advantage over mechanical and hemodynamic indicators in that they are independent of preloads, afterloads, and/or addition of inotropic agents to the KHB solution. Yet, the lack of difference in the MDA concentration may suggest that there is not increased oxidative damage incurred during reanimation of the preserved hearts compared to controls, and that the difference in function is not due to increased oxidative damage during reanimation.

The biochemical analyses described above may also be interpreted to suggest both cardioprotective and antioxidant effects of DHA when added to the preservation solution. However, since there are other differences between these pilot studies, including colloid and lidocaine addition, it is unknown which of the additives may be eliminating or inducing oxidative damage. The fact that pilot study #4 had similar observed function upon reanimation as hearts preserved for longer durations and that it seemed to be more susceptible to ventricular fibrillation suggests that the either the addition of lidocaine or the absence of albumin, DHA, or DADLE could have been considered as proarrhythmic or anti-protective. Yet we acknowledge here that in these pilot studies, the lack of replicates makes it impossible to know which one induced this effect; more studies are planned to better identify such potential interactions.

It is important to consider that given the high cost of performing the described experiments and the modest results to date, it may be worth moving a step back experimentally from whole organ preservation to tissue preservation, e.g., where dissected cardiac muscle bundles are preserved under various CS conditions and subsequently tested for resultant recoverable force productions. Additionally, this would allow for optimization of preservation conditions such as temperature, oxygenation, and preservation solution composition without expensive and labor intensive total heart reanimations. Nevertheless, this tissue bath approach may have a foreseeable confound in that it does not recapitulate the perfusion of the organ, and the potential edema often associated with CHP may not be observed. In such experiments at least the effects of the parameters and additives could be more efficiently estimated than with total heart reanimation.

Conclusion

We report a reproducible experimental approach to assess the outcomes of CHP for optimizing heart preservation. To date, we have observed modestly successful reanimation of swine hearts after extended preservation beyond current clinical methods. In each pilot study, cardiac function was severely diminished in the CHP preserved hearts, and these preserved hearts released higher amounts of troponin and myoglobin into the KHB solution, indicating more cellular damage. However, CHP-preserved hearts exhibited better function than the absent or near absent functionality seen in CS preserved hearts. The addition of various pharmacological pretreatments along with CHP may help to further support subsequent functionalities, e.g., the addition of DHA may induce additive positive effects on cardiac function, biomarkers of cardiac injury, and oxidation. This suggests that our current device and methodology has the potential to extend the duration of heart transplant, but more studies are needed to verify optimization including randomized controlled trials.

Thesis summary

Through the course of this thesis I have investigated cardioprotective treatments with particular focus on the pericardial delivery of antiarrhythmic agents. Through the studies contained herein, it is clear that pericardial delivery provides a unique delivery route that can increase the effects of certain antiarrhythmic agents while decreasing systemic levels and ensuing side-effects.

In order to come to this conclusion, I began with much of the necessary background research, including anatomy and physiology of atrial fibrillation, written up in chapter one. This study of the anatomy compared perfusion fixed hearts with AF to controls, and demonstrated the remodeling that occurs with the disease. I continued on with investigating many different types of targeted drug delivery, in a chapter authored for the textbook Thoracic Surgery. This is reproduced here in chapter two.

The localized delivery of drugs during cardiac surgery and heart transplant posed a unique opportunity for treatment. I began to investigate such deliveries by developing a swine model of AF, first comparing pericardial delivery of metoprolol to IV delivery, then studying the effects of pericardial omega 3 fatty acids with various formulations.

Through this work, I have come to the conclusion that intrapericardial delivery can increase the effectiveness of certain drugs. The enclosed pericardial space increases concentrations of drugs at the targeted site and reduces peripheral exposure. The included experiments were designed to translate into surgical clinical situations where

pericardial access can be easily obtained. The protocols were kept simple to add minimal complexity to heart surgery and transplantation. The timing of the 90 minute treatment was intended to be incorporated into heart transplant procedures, where treatments such as DHA or Lipovenos delivered to the pericardial space during preparation and peripheral organ harvest could help improve hemodynamic performance and reduce postoperative AF in the heart recipient.

Omega-3 polyunsaturated fatty acids have anti-inflammatory and antiapoptotic effects that may contribute to the improved performance observed upon reanimation. This may translate to improved performance of donor hearts upon implantation. With the reduction of tissue damage throughout the transplant procedure, transportation time between donor and recipient may be increased. This would relieve the stress on the logistics of transplantation and allow for increased transport times, likely increasing the number of possible transplants.

The 30 minute timing was designed to fit in with cardiac surgeries that may require some preparation time prior to arresting the heart. For instance, the internal thoracic artery is often used as the donor vessel in a coronary bypass procedure, and this may take 30 minutes to isolate and prepare. This time could be used for treatment with DHA, which was shown in the above experiments to improve function upon reanimation.

These studies are performed in swine, and several steps must take place before widespread clinical application proliferates. Clinical methods for pericardial delivery will require surgical or novel minimally invasive protocols for delivery. To accomplish this,

modifications may need to be made to common surgical techniques and custom tools, such incorporating as the pericardial catheter variations outlined herein. Future steps would include clinical studies to confirm the effective translation into humans prior to use. Such treatments described in detail in this thesis may improve outcomes and increase the total number of heart transplants that are possible.

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Appendix A: Preface

As I started my PhD research, I initial focused on ischemia/reperfusion injury. As the leading cause of morbidity and mortality in the U.S., ischemic heart disease is usually caused by coronary artery disease and myocardial infarction. Ischemia due to reduced blood flow includes a reduction in oxygen, a drop in the availability of nutrients and metabolic substrates, as well as a decrease in waste clearance.

Reperfusion, or the restoration of blood flow, is necessary to minimize cell death due to lack of oxygen, or infarct, and to restore cardiac function. Paradoxically, reperfusion is associated with a host of mechanisms that can lead to further myocardial injury. Mechanisms such as oxygen free radicals, intracellular calcium overload, microvascular and endothelial dysfunction as well as altered metabolic function are mediators of reperfusion injury, and steps taken to mitigate these may reduce damage caused during reperfusion. One such agent that may influence metabolic function is glucagon-like peptide-1 (GLP-1). This appendix contains background and experimental data on certain cardiac effects of GLP-1. This early work served to form much of my foundation of cardiac physiology and pharmacologic treatment. Below is a brief review of GLP-1 and its cardiac effects, as well as a scientific report of the experimental treatments with GLP-1 during cardiac ischemia, which is in preparation for publication.

Glucagon-line peptide-1: Introduction

Glucagon-like peptide 1 (GLP-1) is a naturally occurring incretin hormone secreted by intestinal L-cells in response to ingested nutrients. It stimulates glucose-mediated insulin release, increases insulin sensitivity, mimics response to insulin and inhibits glucagon secretion [1]. Since the insulinotropic actions of GLP-1 are dependent on circulating glucose levels, a major advantage is the reduced risk of hypoglycemia compared to direct injections of insulin. Because of these effects, GLP-1 has been the subject of intense investigation as a therapy for diabetes mellitus, resulting in multiple synthetic versions available for therapy today.

The GLP-1 receptors are widely distributed, located in the stomach, intestinal tract, brain, kidney and heart [1]. Initial studies of the cardiac GLP-1 receptors, along with the classification of a GLP-1 receptor knockout mouse [2], incited interest in the cardiovascular effects of GLP-1. The GLP-1 receptor deficient mice show reduced heart rate and elevated left ventricular (LV) pressures compared to controls at age 2 months. By 5 months of age, the mice lacking the receptor have increased LV wall thickness compared to controls, as well as a lack of a response to intravenous insulin [2]. Thus it is hypothesized that GLP-1 has cardiovascular effects via two routes: cardiac effects mediated through increase insulin secretion and sensitivity as well as direct effects on the myocardium. To that end, GLP-1 has been demonstrated to have its own effects in isolated rodent heart preparations [3–5]. The exact mechanism is not known, but it has been shown that the beneficial effects are abolished by the inhibitors of PI3K, mitogen-activated protein kinase, and cyclic AMP (cAMP), suggesting that these pathways are

involved in the protective response of GLP-1 [4]. The effects can also be blocked by the GLP-1 receptor agonist exendin 9-39.

One of the difficulties with GLP-1 therapy is the short half-life of 1-2 minutes, as the peptide is quickly broken down by dipeptidyl peptidase-IV (DPP-4) [6]. Because of this, several methods for prolonging GLP-1 exposure have been used, including continuous intravenous infusion, subcutaneous delivery, DPP-4 inhibitors and long acting GLP-1 analogs. One such analog was extracted from gila monsters (which eat 5-10 times annually) is Exendin-4, commercialized in synthetic form as Exenatide (Byetta, Amylin Pharmaceuticals), which is injected subcutaneously for diabetic therapy.

Cardiovascular Effects

GLP-1 has been widely studied for its physiological effects on the cardiovascular system and for its therapeutic potential in ischemia and heart failure. GLP-1 and its analogs have been shown to be cardioprotective in rodent [3–5], [7], [8], swine [9], and canine [10] models of ischemia-reperfusion injury.

However, many of the results found in the literature are inconsistent, such as the inotropic effects of GLP-1. Zhao *et al*. showed that GLP-1 treatment resulted in a decrease in left ventricular developed pressure (LVDevP) as well as a decline in LV change in pressure over time (LV dP/dt) in the non-ischemic isolated rat heart [5]. However, when subjected to ischemia/reperfusion injury, GLP-1 improved recovery of LVDevP [5]. Other studies have corroborated these improved hemodynamics after ischemia/reperfusion injury in mice (Ban et al., 2008) and dogs (Nikolaidis et al., 2005) as

well as showing increased cardiomyocyte viability when delivered at reperfusion and/or as a preconditioning mimetic [8], [12]. And Ban and colleagues went on to show that many of these effects are independent of the GLP-1 receptor by producing similar results in a GLP-1 receptor knockout mouse [8], [12]. Ossum and colleagues reported that GLP-1 has a small, non-significant effect on contractility in an ischemia/reperfusion model of the rat heart [13], which could be explained by the cardioprotective property of the treatment. GLP-1 infusion has also demonstrated a significant inotropic effect in a canine model of heart failure [14], [15].

When studied in acute infarction models, GLP-1 has been suggested to reduce tissue damage when administered as a preconditioning agent or at reperfusion. Bose *et al*. showed GLP-1 was effective at reducing infarct size when co-administered with the DPP-4 inhibitor valine pyrrolidide (VP), both as a preconditioning mimetic and during the first 15 minutes of reperfusion in isolated rat hearts[3], [4], [7] and in vivo [4], but did not reduce infarct size in the absence of the DPP-4 inhibitor [3]. Kavianipour et al. [16] reported no effect on hemodynamics or infarct size when GLP-1(7-36) amide was administered intravenously in pigs before and during ischemia/reperfusion. But Timmers *et al*. demonstrated in a swine model that injections of Exenatide five minutes prior to reperfusion and on the subsequent two days reduced the infarct size and deterioration in cardiac function observed in the control group [9].

In addition to GLP-1 in its entirety, more specifically known as GLP-1(7-36), the effects of the initial breakdown product, GLP-1(9-36), have also been studied. Cardioprotective effects of GLP-1(9-36) remain controversial with observed reduced infarct size in mouse

hearts [17] but no effect seen in rat hearts [13]. Additionally, GLP-1(9-36) has negative

inotropic effect on the isolated rat heart [13], but positive inotropic effects in dogs with

dilated cardiomyopathy [15]. Another study showed pretreatment with the metabolite

GLP-1(9-36) amide actually worsened the hemodynamics compared to controls, while

administration on reperfusion had a positive effect on both hemodynamics and infarct

size [8]. Reports such as these in the literature suggest that GLP-1(9-36) amide may act

independent of the GLP-1 receptor to produce cardioprotective effects when delivered

initially upon reperfusion [8], [12], [18]. Unless otherwise noted, the use of "GLP-1"

refers to the GLP-1(7-36) moiety. Throughout the study of GLP-1, the particular analog

chosen, administration with or without inhibitor, timing, dose and duration of delivery,

and the species and model used to evaluate the peptides for their therapeutic potential

are all have great influence on the observed response.

In vitro trabeculae model

In order to study the direct effects of GLP-1 on contractility in a time and cost efficient

manner, I use an *in vitro* trabeculae model for some of the studies in Chapter 1. This

model uses trabeculae carnae dissected off the endocardial surface of porcine (or other

large mammalian) hearts. This model has numerous advantages over *in vivo* studies,

such as the isolating the effects of GLP-1 from other systemic effects including insulin

release and sensitivity, as well as other hormonal and autonomic responses. This is

beneficial since GLP-1 is hypothesized to have direct effects on cardiac function,

independent of its insulinotropic effects [3]; however, when delivered intravenously or

subcutaneously *in vivo*, all systemic effects of the treatment will be generated.

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Other advantages of this model include reduced time and cost. The equipment in our lab allows a single heart to be used in up to 22 studies to be performed simultaneously. Thus, tissue from a single animal can be used in several unique treatments as well as serve as its own controls. The *in vitro* approach also uses only a small amount of buffer. This allows precise control over dosing as well as requiring only a small amount of drug compared to a whole animal or Visible Heart® preparation [19].

Another advantage specific to the use of GLP-1 in the isolated trabeculae model is its increased half-life. When administered intravenously *in vivo*, GLP-1 has a half-life of 1-2 minutes, as the peptide is quickly broken down by DPP-4 [10]. When administered in the isolated tissue model, in the absence of DPP-4, the half-life is significantly extended, thus removing the need for a continuous infusion. In pilot studies, the half-life was measured to be over 35 minutes.

The drawbacks to this model include the artificial environment that the bundles are placed in. Additionally, the tissue necessarily goes through a short period of hypoxia before the bundles are hung. The damage is minimized by cooling the tissue and removing any form of stimulation to help reduce metabolic demand.

Ventricular trabeculae were chosen for convenience and consistency. In addition to being appropriately sized, each trabecula is easy to dissect from the endocardium with minimal damage to the myocytes. Additionally, fiber orientation within the myocardial wall is not always clear and changes with depth. Using trabeculae maximizes longitudinal fiber orientation, which is desirable for maximum contractility.

This model also allowed for the measurements of the direct effects on human myocardium. Using a unique connection with the University of Minnesota Fairview hospital, we were able to acquire human ventricular tissue from heart transplant recipients. This provided a unique opportunity to translate protocols to human myocardium in heart failure. Combined with our isolated trabeculae model, we were able to efficiently evaluate the effects of GLP-1 on swine and human tissue.

This trabeculae model was used to investigate the inotropic effects of GLP-1 and Exendin-4, as well as any protective effects during hypoxia/reperfusion injury by measuring contractility effects on swine tissue. After 11 pig hearts and 178 trabeculae (63 non-hypoxic, 115 hypoxic), no inotropic effect was seen in the non-hypoxic model and no improvements in tissue function after hypoxia/reperfusion injury were seen at two dosing levels of each GLP-1 or exendin-4 (details in chapter 1).

During the course of this study, we received 4 human heart biopsies and were able to test 34 trabeculae. The failing heart dropped in function in throughout the experiments, losing 80% of its function regardless of treatment level or even exposure to hypoxia/reperfusion (Figure A.1). This is likely due to the severe heart failure of the individual hearts. Not only did GLP-1 not have an effect at either dose tested, the hypoxia/reperfusion protocol did not damage tissue any more than being *in vitro* for four hours as seen by comparing to the non-hypoxic trabeculae. This same hypoxia/reperfusion protocol reduced function approximately 30% compared to non-

hypoxic porcine tissue (see study, below). The dramatic drop in function in all contractile force makes it difficult to draw conclusion from this human model.
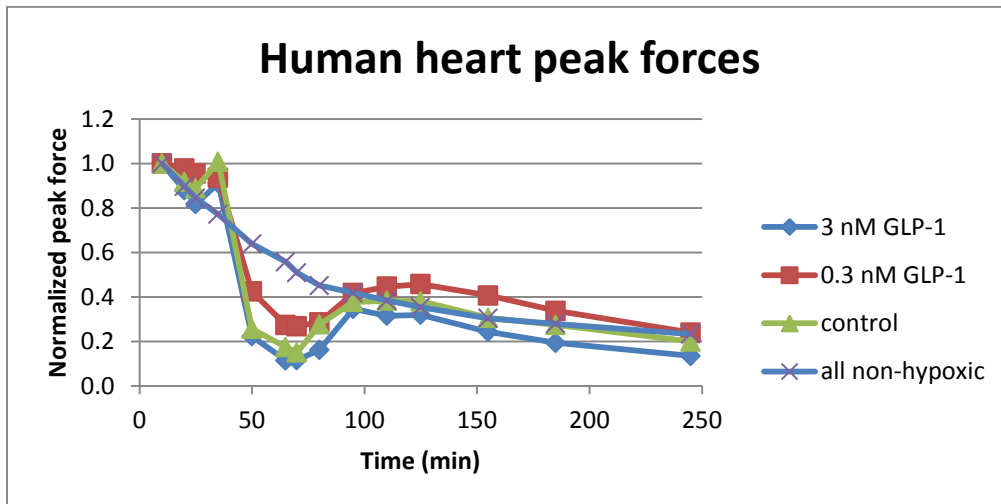

Figure A.1: peak forces of human LV trabeculae. Peak forces dropped throughout the experiment to approximately 20% original function.

Delivery method

While most studies using GLP-1 deliver intravenously (IV) or directly to the coronary arteries in isolated *in vitro* models, subcutaneous (SC) delivery offers a slower absorption rate. In fact, the clinically approved analogs, Byetta and Bydureon, are administered SC for diabetic therapy. For SC delivery, pigs are most similar to humans in terms of skin and subcutaneous fat. In contrast to rats and dogs, pigs exhibit similar absorption rate of insulin into the plasma after SC delivery compared to humans [20]. The possibility of SC delivery of GLP-1 was explored in comparison to IV delivery in a pilot study in our lab. The main advantage of a SC bolus upon reperfusion compared to continuous IV infusion is the potential to simplify the delivery method in a clinical setting.

For our studies, we used the GLP-1(7-36)amide, as there is more evidence of clinical

relevance compared to the breakdown product GLP-1(9-36)amide. Henceforth, any

reference of GLP-1 is to the GLP-1(7-36)amide, unless otherwise noted.

The objectives of this pilot study were to compare the pharmacokinetic actions of GLP-1

delivered IV vs. SC. The specific delivery protocols are outlined below

A) Continuous IV Dose-Escalation: Three doses (1.5, 3.0, 4.5 pmol/kg/min) of
   GLP-1 were delivered consecutively via continuous IV infusion for 30 min
   each, followed by a 60-min washout period. (n=2)
B) Continuous SC Dose-Escalation: Three doses (1.5, 3.0, 4.5 pmol/kg/min) of
   GLP-1 were delivered consecutively via continuous SC infusion for the same
   duration, followed by a second washout period.   (n=2).
C) Bolus SC injections at two increased doses (0.27 and 2.7 nmol/kg/min) with
   appropriate washout between. (n=2)
D) Continuous IV infusion at the lowest dose (1.5 pmol/kg/min) for twice the
   duration (60 min) (n=1)

Because of the short half-life of GLP-1 in the blood, it was decided that delivery

protocols A and B could be performed in the same animals with the included 60 minute

wash out period. Both protocol A and B were performed in pig 1 and pig 2.   After

inconsistent results with SC delivery (see Figure A.3), modifications in the protocol were

made in an attempt to troubleshoot the lack of a dose-escalation response. Possible

explanations for ineffective delivery included inappropriate site and too small of a dose.

Saline was used as buffer in the first two experiments, and the Amylin buffer was used

for subsequent experiments. In addition to the increased dosing for part C, the site was

changed based on the literature [20]. Subcutaneous delivery was simplified by doing

bolus injections rather than infusions. In addition, a longer infusion was added (protocol

D) to confirm stability, which was performed in the last animal prior to SC delivery.

Four male Yorkshire swine weighing 72.2 – 99.8 kg were anesthetized for these

pharmacokinetic experiments. A femoral artery was cannulated for measurement of

blood pressure and withdrawal of blood samples, which were drawn every 10 minutes and

processed via ELISA (LINCO Research, St. Charles, MI) to measure active GLP-1

plasma levels.

For intravenous delivery, GLP-1 was infused through the Swan-Ganz catheter into the

right ventricular outflow tract. For subcutaneous delivery, a 25-gauge needle was

inserted at a 45-degree angle to pinched skin at the sites shown in Figure A.2.
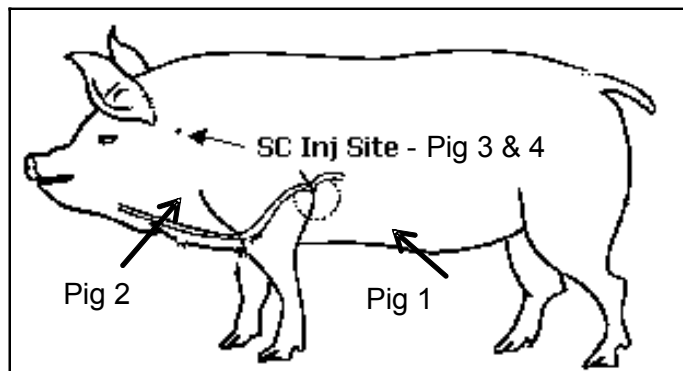
Figure A.2: Sites for subcutaneous delivery site in Pigs 1-4. After no plasma GLP-1 was detected in the first experiment, the site was changed to avoid regions of deep subcutaneous fat (the abdomen) to optimize delivery. The targeted regions were the neck/shoulder [20], [21] **in Pig 2 and behind the ears in Pig 3 and 4.**

Pilot Results

A repeatable dose-dependent elevation in plasma levels were observed during intravenous infusion in the dose-escalation experiments (Pigs 1 and 2, Figure A.3). With subcutaneous infusions, however, there was moderate but inconsistent elevation in Pig 1, and no appreciable elevations in Pig 2.
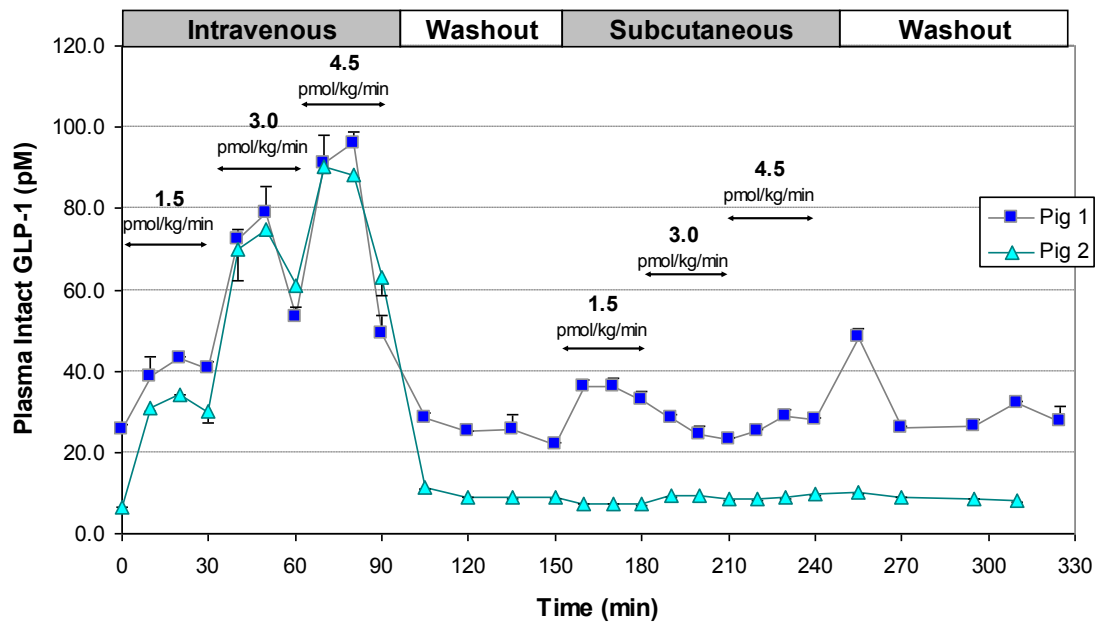
Figure A.3 Plasma levels of intact GLP-1 in Pigs 1 and 2 over time in a dose-escalation study at three doses (1.5, 3.0 and 4.5 pmol/kg/min) delivered 30 minutes each with a 60-minute washout period between IV and SC delivery.

Figure A.4 shows active GLP-1 plasma levels in Pigs 3 and 4 over time. In Pig 3, two bolus injections were given at 0.27 and 2.7 nmol/kg. Elevated levels of GLP-1 were detected in the plasma for both injections at two doses. For the 0.27 nmol/kg dose, the maximum plasma level was reached at 10 minutes and showed an exponential decay to baseline shortly after injection. The 2.7 nmol/kg dose shows a more gradual decay with

a sudden spike 50 minutes post-injection, and plasma levels remained elevated at the conclusion of the experiment 60 min post-injection.   In Pig 4, stable plasma levels were achieved with intravenous infusion. No elevation was seen with the low dose bolus, and compared to Pig 3, the plasma levels decayed more quickly with the high dose bolus.
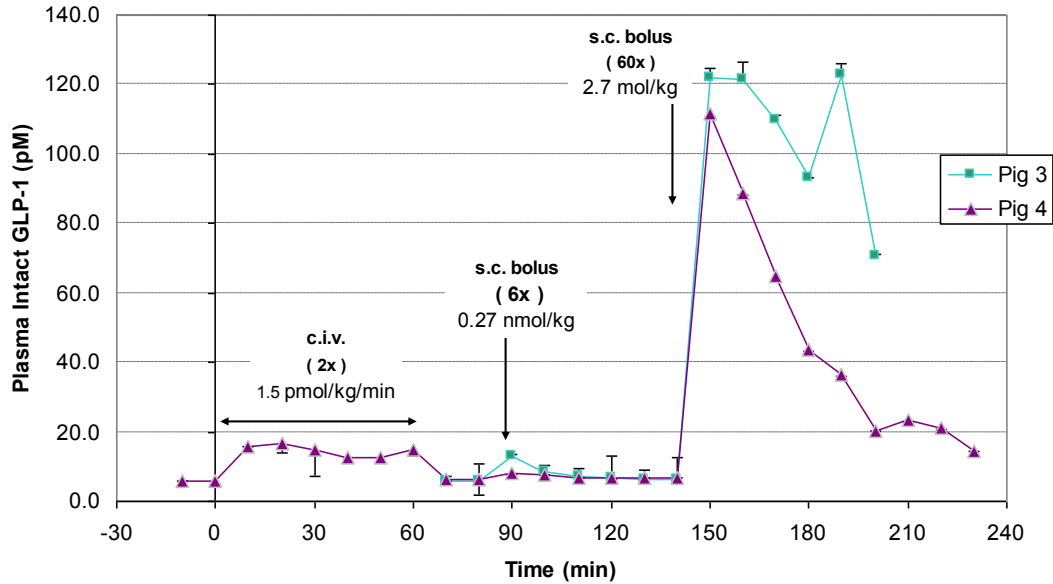
Figure A.4: Plasma levels of intact GLP-1 in Pigs 3 and 4 over time with 60 minutes of IV infusion at 1.5 pmol/kg/min and a 30-minute washout period in Pig 4 only and two SC bolus doses with a 60-minute washout period between boluses in both Pigs 3 and 4.

Interpretation

In these experiments, plasma GLP-1 concentrations at expected levels were consistently achieved with intravenous administration, while subcutaneous administration yielded inconsistent or undetectable results with both infusion and bolus delivery. In light of these results, further work completed with GLP-1 in swine was delivered IV. It was decided that the consistency and predictability afforded by IV delivery outweighed the potential clinical benefits of SC delivery.

The study in Chapter 1 makes use of the *in vitro* trabeculae preparation to efficiently evaluate the inotropic effects of GLP-1 and Exendin-4 in the absence of systemic effects. A corresponding trabeculae study tests the preconditioning and cardioprotective effects of GLP-1 and Exendin-4 in tissue subjected to hypoxia/reperfusion injury. The lack of effect seen *in vitro* suggests that there is little to no direct effect in this model.

An accompanying swine *in vivo* model of acute myocardial infarction was also used. In this open-chest model, the left anterior descending coronary artery was occluded for 45 minutes followed by reperfusion for 180 minutes. Either GLP-1 or Exendin-4 was infused for the first 30 minutes of reperfusion. The main finding was that Exendin-4, but not GLP-1, significantly reduced the size of induced infarct.

Pigs were chosen for this study based on their cardiac and coronary anatomy. Pigs are a good model since their coronary vasculature is similar to humans. The open chest model provides a reliable and repeatable model that ensures proper occlusion and reperfusion.

In preparation for publication: The potential utilities of glucagon-like peptide-1 and exendin-4 in a porcine acute ischemia reperfusion model

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Summary

Background: GLP-1 (glucagon-like peptide 1) is an incretin hormone secreted by intestinal L-cells in response to ingested nutrients. It stimulates insulin release, increases insulin sensitivity, mimics response to insulin, inhibits glucagon secretion, decreases norepinephrine, and improves cell survival. GLP-1 has been widely studied for its physiological effects on the cardiovascular system and for its therapeutic potential in ischemia and heart failure. GLP-1 and its analogs have been shown to be cardioprotective in rodent [3–5], [7], [22], swine [9], and canine [10] models of ischemia-reperfusion injury. The long-acting analog exendin-4 has the potential to provide similar benefits in a more stable form.

Methods: In vitro experiments were performed with isolated trabeculae from porcine hearts. GLP-1 and exendin-4 were administered to test direct effects on contractility and performance after hypoxia/reperfusion injury. A model of acute myocardial infarction (AMI) was used in eight Yorkshire swine divided into two treatment groups receiving GLP-1(7-36) or exendin-4. Historical controls from previous infarct experiments [23] at the same laboratory were used as controls for this study. After median sternotomy and stabilization, the left anterior descending (LAD) coronary artery was occluded for 45 minutes. Therapy was started two minutes prior to reperfusion and continued 30 minutes into the 180 minute reperfusion period. Blood samples were drawn for metabolic assessment of myocardial glucose and lactate, insulin, and the administered peptides throughout reperfusion. At the end of reperfusion, the LAD was re-occluded and patent blue dye injected into the right ventricle to differentiate the area subjected to

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ischemia. The heart was harvested, frozen, sectioned and stained with triphenyltetrazolium chloride to determine area at risk and infarct areas.

Results: In isolated trabeculae, neither GLP-1 nor exendin-4 had significant effects on contractility in either the non-hypoxic or hypoxic model. In vivo, intravenous administration of Exendin-4, but not GLP-1(7-36) amide, significantly reduced infarct size in this small investigational study (55.4 ± 15.2% for GLP-1; 48.7 ± 19.7% for Control; 18.3±12.4% for exendin-4). Either GLP-1 or Exendin-4 was detected in the plasma of the respective treatment groups, confirming efficacious delivery of the peptides. One or more episodes of ventricular fibrillation occurred in 50% of the Exendin-4 group, 25% of the control group, and 0% of the GLP-1 group. There were no mortalities in either treatment group, while the control group experienced 17% mortality [23]. GLP-1 had no significant effect on hemodynamics, but exendin-4 increase arterial mean and diastolic pressures. Plasma insulin increased during the treatment period in 2 of the 3 animals tested, one in the GLP-1 group and one the Exendin-4 group, which may be due to administration of these insulinotropic peptides

Conclusions: Exendin-4 but not GLP-1 reduced infarct size in this small study. Beneficial effects may in part be explained by the increase in arterial pressure in the exendin-4 animals. Further preclinical studies are warranted to determine dose, analog, and heart failure population best served by continuous infusion of exendin-4.

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Introduction

Glucagon-like peptide 1 (GLP-1) is a naturally occurring incretin hormone secreted by the gut after meals and is responsible for glucose-mediated insulin release [1]. Because of its insulinotropic and insulinomimetic properties, as well as evidence of GLP-1 receptors on the heart, GLP-1 has been investigated for its cardioprotective effect and its role in cardiac physiology [1–4], [11].

In various animal and clinical studies, numerous administration protocols have been employed. Because the active form of GLP-1 (7-36)amide is quickly broken down by dipeptidyl peptidase-4 (DPP-4), several methods for prolonging GLP-1 exposure have been used, including continuous infusion, DPP-4 inhibitors and long acting GLP-1 analogs such as Exendin-4.

The hemodynamic and cardioprotective effects of GLP-1 are not consistent in the literature. Delivery of GLP-1(7-36) amide has resulted in improved hemodynamics after ischemia/reperfusion injury [8], [10], [11] and increased cardiomyocyte viability when delivered at reperfusion and/or as a preconditioning mimetic [8], [12]. Ferthermore, Ban and colleagues went on to show that many of these effects are independent of the GLP-1 receptor by producing similar results in a GLP-1 receptor knockout mouse [8], [12].

Bose et al. [7] showed GLP-1(7-36) amide was effective at reducing infarct size in isolated rat hearts when co-administered with the DPP-4 inhibitor valine pyrrolidide (VP), both as a preconditioning mimetic and during the first 15 minutes of reperfusion. When delivered during stabilization and throughout ischemia/reperfusion, GLP-1 reduced

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infarct size in rat hearts when co-administered with VP both in vivo [4] and in isolated rat hearts[3], [4] but did not reduce infarct size in the absence of the DPP-IV inhibitor[3]. Kavianipour et al. [16] reported no effect on hemodynamics or infarct size when GLP-1 was administered intravenously in pigs before and during ischemia/reperfusion. But with the synthetic Exendin-4, Exenatide, Timmers *et al*. demonstrated in a swine model that injections 5 minutes prior to reperfusion and on the subsequent 2 days reduced the infarct size and deterioration in cardiac function observed in the control group [9]. Clearly, the particular analog chosen, administration with or without inhibitor, timing, dose and duration of delivery, and the species and model used to evaluate the peptides for their therapeutic potential are variables affecting the observed response.

This study measures the effects of GLP-1 and exendin-4 on two porcine ischemia/reperfusion models. The direct inotropic and preconditioning effects are tested *in vitro* using porcine myocardial tissue, while the cardioprotective effects are studied in an open chest swine model of acute myocardial infarction.

Methods
Peptide preparation

Lyophilized GLP-1 and Exendin-4 were obtained in 1 mg quantities from Bachem Americas, Inc. (Torrance, CA). Dry peptides were reconstituted and aliquoted into 2 mL quantities for long-term storage at -20C. Two solvents were used for this purpose: 1) modified Krebs solution, and 2) "Amylin" solution. The compositions of these solvents are described in Table A.1.

Krebs solution		Amylin solution		
Ingredient	Quantity	Ingredient	Unit Formula (mg/mL)	Function
NaCl	118 mM	Mannitol	50.7 mg	Osmotic Agent
D-Mannitol	16 mM	Glacial Acetic Acid	0.38 mg	Buffer
D-Glucose	5.75 mM	Sodium acetate trihydrate	0.49 mg	Buffer
$NaHCO_3$	20 mM	Water for Injection	qs to 1.0 mL	Solvent
2Na-EDTA $\cdot$ $2H_2O$	0.32 mM			
KCl	4.5 mM			
$MgCl_2 \cdot 6H_2O$	1.455 mM			
$NaH_2PO_4 \cdot H_2O$	1.2 mM			
$CaCl_2 \cdot 2H_2O$	1.81 mM			
1% Bovine Serum Albumin				

Table A.1: Contents of the Krebs and Amylin solutions used as solvents for drug compounds

Isolated trabeculae model

Swine hearts were harvested within 10 minutes of sacrifice from animals used for other non-phamacological studies and prepared as previously described [24]. Briefly, trabeculae carnae 1-2 cm in length and 2-3 mm in diameter were dissected off the endocardium and hung on a calibrated force transducer, then mounted in an experimental chamber kept at 37° C. The chamber was filled with a krebs henseleit buffer and gassed with carbogen (95% O_2, 5% CO_2). The buffer composition consisted of: NaCl (118.0mM), D-Mannitol (16.0mM), D-Glucose (5.75mM), $NaHCO_3$ (20.0mM), 2Na-EDTA$\cdot$$2H_2O$ (0.32mM), KCl (4.5mM), MgCl2$\cdot$$6H_2O$ (1.46mM), $NaH_2PO_4$$\cdot$$H_2O$

(1.2mM), and CaCl$_2$ (1.81mM). The trabeculae were stimulated with a 1ms 15V pulse

(pulse width of 1ms) at 0.1 Hz The transducer signal was amplified and recorded in a

LabView program (National Instruments, Austin TX).   They were subjected to a

hypoxia/reperfusion protocol outlined in Figure A.5, and dosing in Table A.2.

	Hearts	Hypoxia/Reperfusion			Non-Hypoxic		
		3.0nM	0.3nM	Control	3.0nM	0.3nM	Control
GLP-1	7	26	28	27	11	12	13
Exendin-4	4	13	11	10	10	9	8

Table A.2: Total number of swine and human hearts studied, as well as the number of trabeculae at each dose.
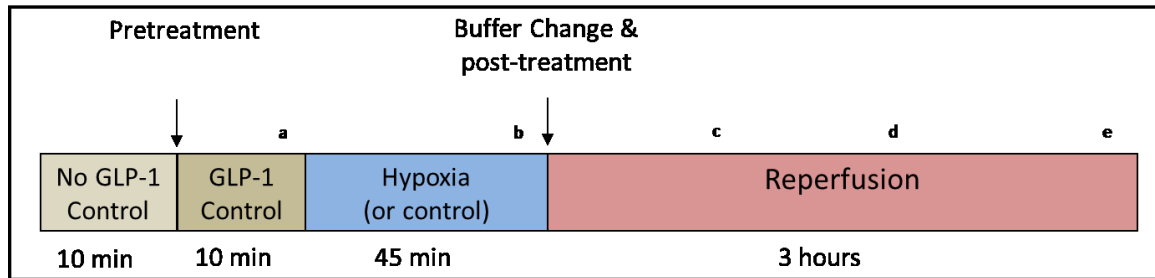
Figure A.5: Hypoxia study timeline. Buffer samples are drawn for analysis at points marked a-e.

Acute Myocardial infarction

Animals

Approval for these animal experiments was granted from the Institutional Animal Care

and Use Committee of the University of Minnesota. Eight Yorkshire swine weighing 44.3

± 3.2 kg were divided into two treatment groups to receive either GLP-1(7-36) amide (n=4)

or GLP-1 receptor agonist exendin-4 (n=4). Historical controls from previous infarct

experiments [23] at the same laboratory were used as controls for this study (n=6).

Surgical Procedure

The anesthesia and surgical procedures are very similar to those described previously in the papers of Xiao [23] and Sigg [23], [25], [26]. Briefly, Yorkshire swine weighing 44.3 ± 3.2 kg were anesthetized and instrumented. Two Millar Mikro-Tip pressure sensor catheters (5 Fr, model MPC-500) were placed, one in each ventricle. A Swan-Ganz thermodilution catheter (Edwards Lifesciences; Irvine, CA) was placed in the pulmonary artery and connected up to a Q2 Continuous Cardiac Output Computer (Abbott Laboratories; Abbott Park, IL) to monitor continuous cardiac output. Blood pressure and samples for blood gas analysis were drawn through a cannulated femoral artery.

A median sternotomy was performed and the heart suspended via a four-suture pericardial cradle. A 2- to 3-mm segment of the LAD coronary artery was dissected distal to the first diagonal branch (between second and third branch) for occlusion. The azygos vein was clamped and the coronary sinus cannulated for venous blood sampling.

The animal was heparinized (300 IU/kg IV bolus of heparin followed by an infusion of 67 IU/kg/hr) and allowed to stabilize for one hour before starting the experimental protocol. To induce ischemia, a vessel clamp (Sklar Vascular Size 2 Single Clamp) was placed on the LAD between the second and third branch for 45 minutes. Treatment was started 2 min prior to reperfusion according to one of the three dosing schemes illustrated in Figure A.6 and summarized in Table A.3. Peptides were delivered into the right ventricle at 1.0 mL/min through the proximal port of a Swan-Ganz catheter connected to a Harvard syringe pump. The reperfusion period lasted for 180 min.
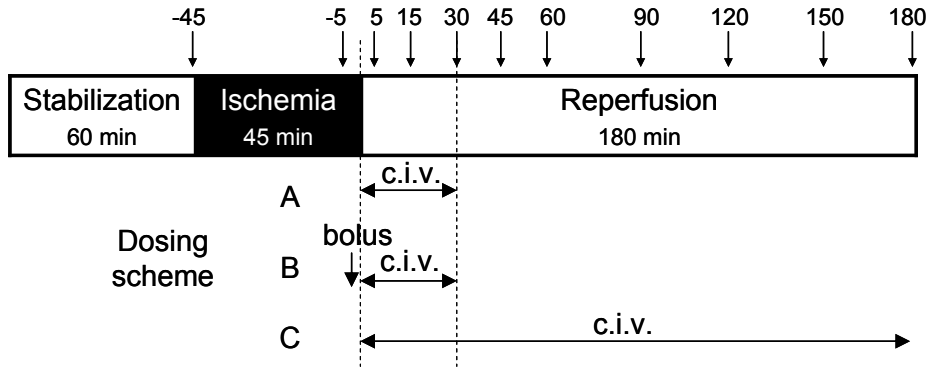
Figure A.6: Timeline of the experimental protocol, blood draws, and dosing schemes. c.i.v: continuous IV delivery. Bolus: SC bolus

Animal #	Treatment						
	Analog	Formulation	Total dose (pmol/kg)	Dosing protocol	Loading dose (pmol/kg)	Infusion rate (pmol/kg/min)	Duration (min)
1	GLP-1(7-36)	Saline	45	A	-	1.5	30
2	GLP-1(7-36)	Saline	45	A	-	1.5	30
3	GLP-1(7-36)	Saline	45	A	-	1.5	30
8	GLP-1(7-36)	Amylin	450	C	-	2.5	180
4	Exendin-4	Amylin	45	A	-	1.5	30
5	Exendin-4	Amylin	45	A	-	1.5	30
6	Exendin-4	Amylin	45	B	30	0.5	30
7	Exendin-4	Amylin	45	B	30	0.5	30

Table A.3: Summary of GLP-1 analog, formulation, and dosing scheme for each animal

Electrocardiograms, right and left ventricular pressures, ascending aortic flow, and arterial blood pressure were recorded simultaneously with IOX software (EMKA Technologies, Falls Church, VA) and BIOPAC MP150 (BIOPAC Systems, Inc., Goleta, CA) data acquisition systems. Derived parameters heart rate, LV dP/dt max, LV systolic pressure, LV end-diastolic pressure, LV developed pressure, relaxation time constant, and mean arterial pressure were also calculated. Blood samples were acquired at the time points shown in Figure A.6. Arterial samples were analyzed for blood gases and plasma levels of insulin, glucose, lactate, GLP-1, its primary metabolite GLP-1(9-36) and Exendin-4. Venous samples were also analyzed for blood gases, glucose, and lactate.

Continuous cardiac output was recorded via the thermodilution catheter at regular intervals and at the time of blood draws. Arrhythmia assessment occurred throughout ischemia and during the first 45 minutes of reperfusion.

Determination of Infarct Size and Risk Area

At the conclusion of the 180-minute reperfusion period, the LAD was reoccluded and patent blue dye injected into the right ventricle to differentiate the ischemia area (area at risk) from the non-ischemic area. Immediately following sacrifice, the heart was harvested *in toto*, rinsed, and stored at -20°C for 14-18 hours. The frozen hearts were sliced into 4-mm transverse sections and stained with 1% triphenyltetrazolium chloride (TTC). Slices were photographed and analyzed to determine relative sizes of infarct size and area at risk.

Arrhythmia Assessment

Blinded analysis of arrhythmias was performed by the same person who performed the assessment in historical controls using the following scoring system: $0 = \leq 10$ premature ventricular contractions (PVCs) in 9 min; $1 = 11–50$ PVCs in 9 min; $2 = > 50$ PVCs in 9 min; $3 = 1$ episode of ventricular fibrillation (VF) in 9 min; $4 = 2–5$ episodes of VF in 9 min; and $5 = >5$ episodes of VF in 9 min. When VF was elicited, cardioversion was attempted using 30-J shocks administered via internal paddles and repeated as necessary.

Plasma Sampling and Analysis

Blood samples were collected in ice-cooled Vacutainer EDTA-plasma tubes containing 10 µl of 10 mM DPP-IV (Millipore Corporation; St. Charles, Missouri) per mL of blood when necessary. Within the hour, samples centrifuged and analyzed for plasma glucose and lactate (Yellow Springs Instruments Model 2300 Stat Plus glucose/lactate analyzer) and aliquoted and stored at -80°C. ELISAs were used to measure intact GLP-1, i.e. GLP-1 (7-36) amide and GLP-1 (7-37), as well as total GLP-1 and Exendin-4 where appropriate (Assays from LINCO Research, Phoenix Pharmaceuticals, Inc.). Insulin was measured in Pigs 3, 4 and 5 with ELISA (ALPCO).

Statistical Analysis

All results are expressed as the mean ± SD. Data were compared used one-way ANOVA with MEDSTAT for Excel v 5.2 software. When the F value was significant, individual group means were compared using the Newman-Keuls method for multiple contrasts in GraphPad Prism 5 software. When control data were not available, an unpaired Student's t-test was used to compare treatment groups. A P value of <0.05 was considered significant.

Results
Isolated trabeculae model

The half-life of GLP-1(7-36) was extended by the lack of DPP-4 *in vitro*, with 50-65% percent remaining at 30 minutes (average observed half-life 35.6 min). Non-hypoxic tissue slowly declined in function over the course of the 4 hour experiment, to approximately 80% of initial function. Treatment with GLP-1 or exendin-4 throughout the 4 hours had no effect on force production (Figure A.7 and Figure A.8). During hypoxia,

the forces produced by all trabeculae fell to about 40% of their initial value, then

recovered to 50-60% during reperfusion. There is very little difference among the

groups with no statistically significant differences (Figure A.9 and Figure A.10).
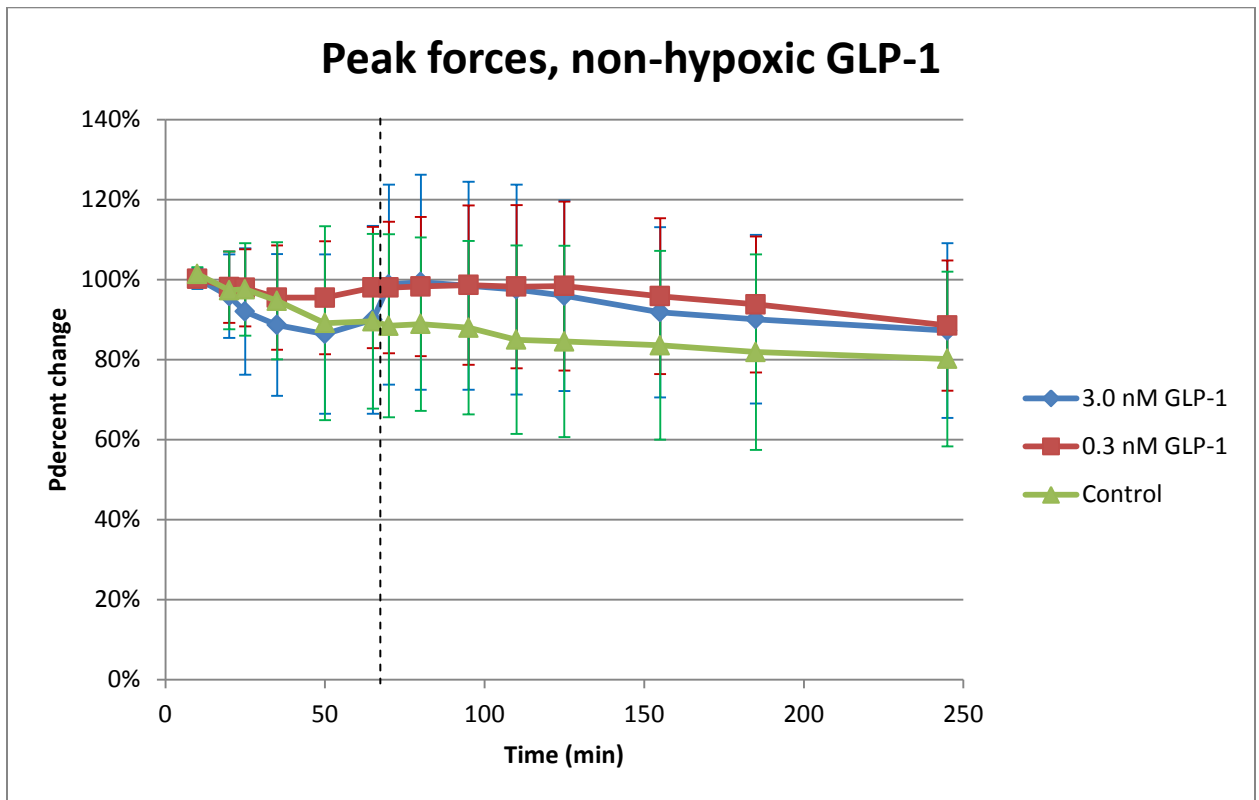

Figure A.7: Average of peak forces from each dose of GLP-1 relative to baseline. The vertical dotted line indicates a buffer change. GLP-1 was in the buffer throughout the entire experiment.
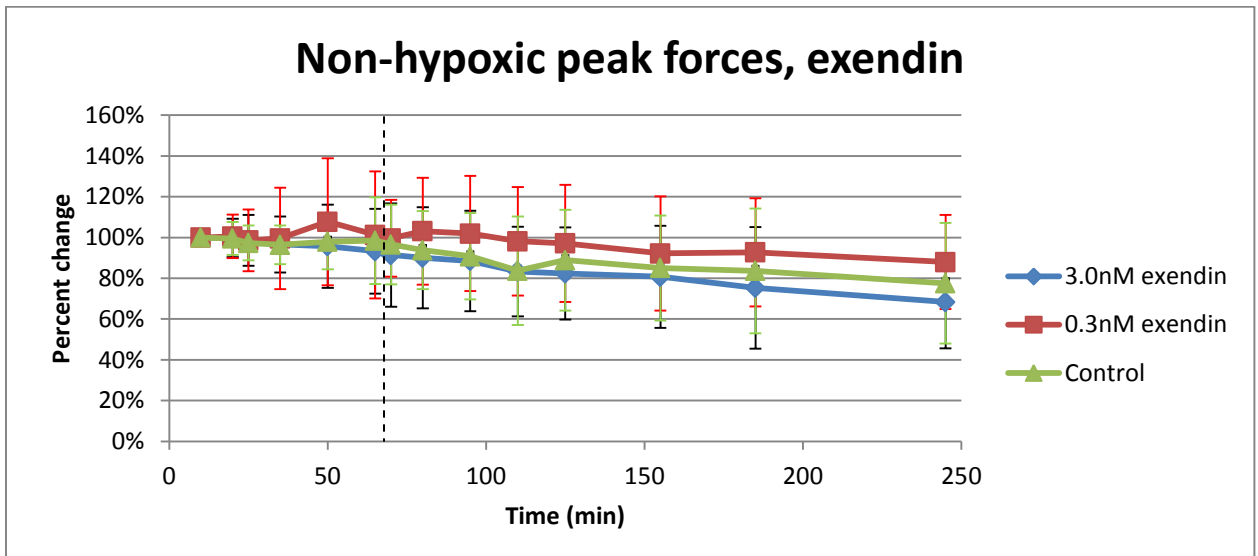
Figure A.8: Average of all peak forces from each dose of exendin-4 in trabeculae not subjected to hypoxia. The vertical dotted line indicates a buffer change. Exendin-4 was in the buffer throughout the entire experiment.
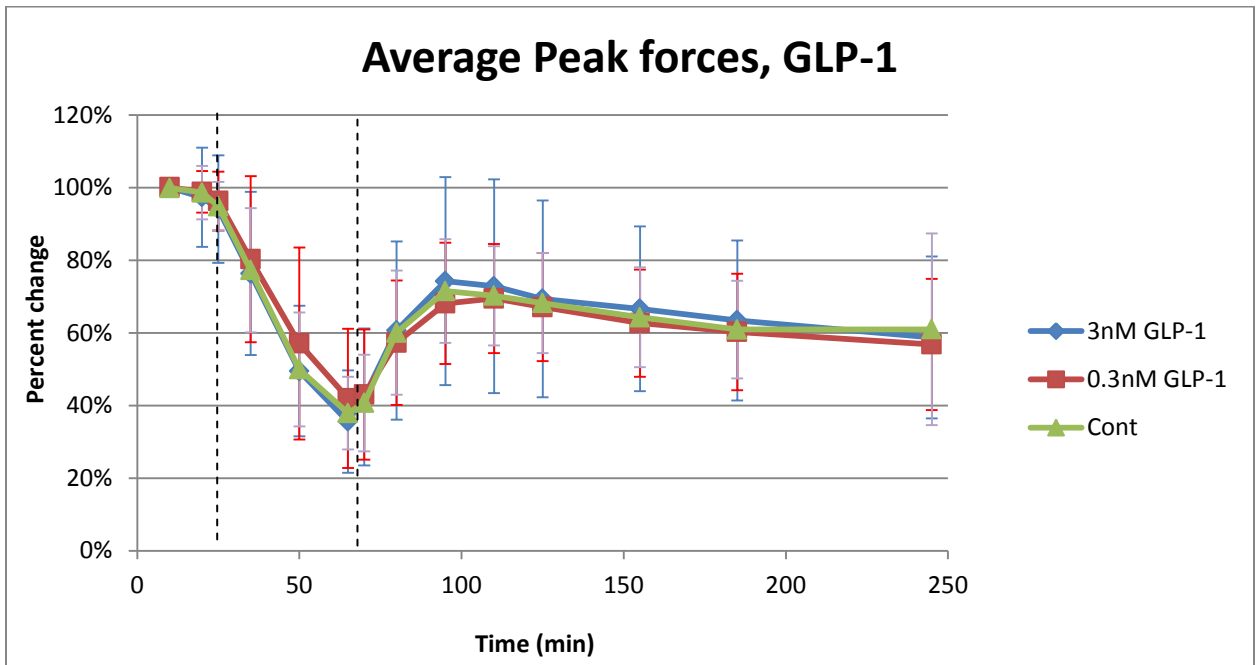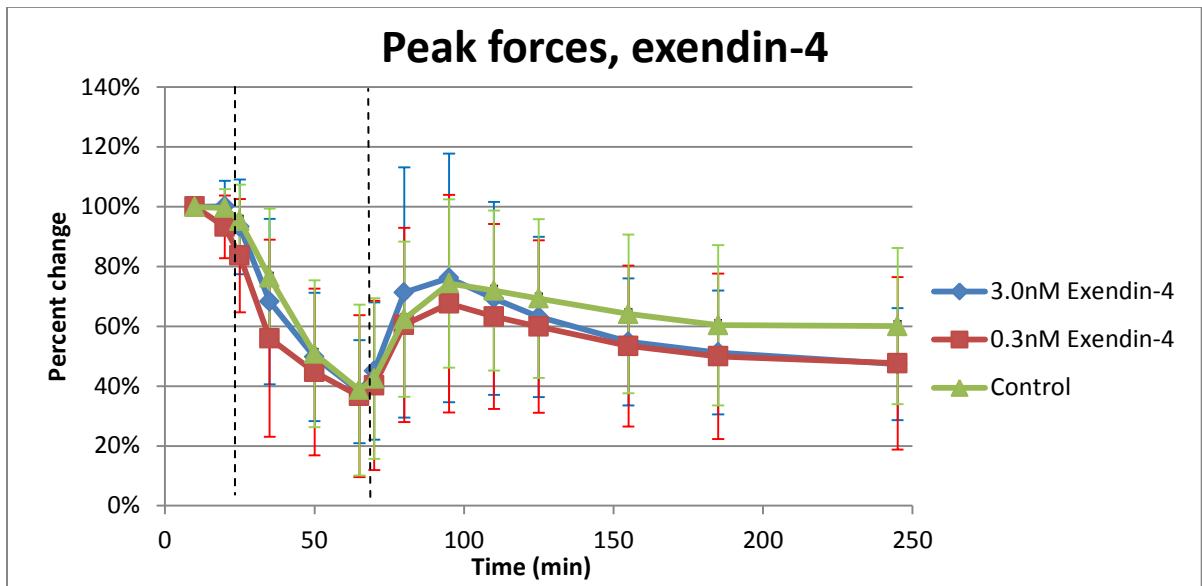

Figure A.9: Effects of GLP1 on porcine trabeculae subjected to hypoxia/reperfusion injury. Tissue was hypoxic during time between hashed vertical lines.

Figure A.10: Average peak force from trabeculae treated with Exendin-4. Tissue was hypoxic between vertical hashed lines.

The rate of force development and decay were calculated as measures of contractility and relaxation. Furthermore, baseline forces were measured as an indicator of preload. No differences were seen among any of these these groups (data not shown). Tissue bath lactic acid levels were also measured in both the GLP-1 groups and the exendin-4 groups at various points. The levels of lactic acid increased through the experiment and there were no significant differences between the treatment groups during the hypoxic or non-hypoxic time points.

Infarct Size

In the acute MI model, the LAD was occluded for 45 minutes, and either GLP-1 or Exendin-4 was given just prior to 180 minutes of reperfusion. The area at risk, or

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ischemic zone, was similar among all groups: 18.4 ± 3.3% in the GLP-1 group, 19.0 ± 7.7% in the Exendin-4 group, and 22.7 ± 8.1% in the control group. As a percent of the total area at risk, the infarct area was not significantly different between the GLP-1 and control groups (55.4 ± 15.2% vs. 48.7 ± 19.7%). However, infarct size was significantly reduced in the Exendin-4 group (18.3±12.4%) compared to both the GLP-1 and control groups (p<0.01 and p=0.03, respectively; see Figure A.11).
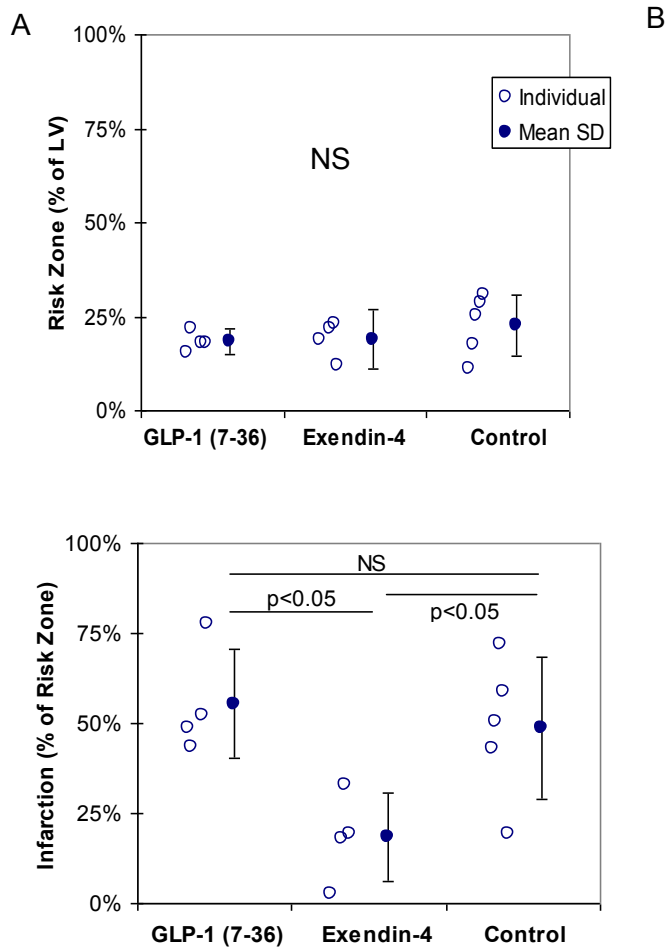
Figure A.11: (A) Risk Zone, the percent of LV subjected to ischemia, and (B) Infarct Area, the percent of the risk zone that is infracted, for treatment and control groups.

Arrhythmias

There were no occurrences of VF in the GLP-1 group. Ventricular fibrillation occurred in two animals in the Exendin-4 group, one instance in Pig 5 and five instances in Pig 7. No mortalities resulted from ventricular fibrillation in either group after defibrillation. Morbidity and mortality from VF in the historical control group was 25% and 17%, respectively. There was no difference in the total arrhythmia score for each treatment group compared to control or between treatment groups during the reperfusion period (Figure A.12) as well as during the ischemic period (not shown).
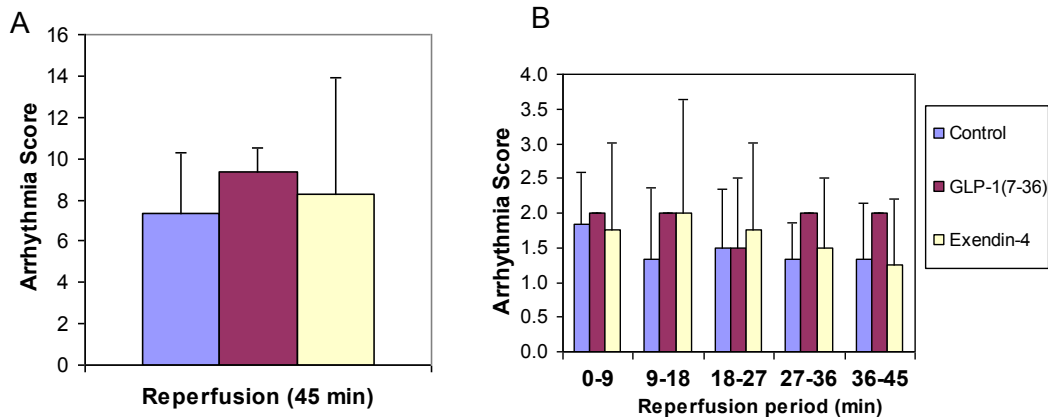
Figure A.12: Arrhythmia score (A) over the 45-minute reperfusion period and (B) broken down over each 9-minute segment of the reperfusion period.
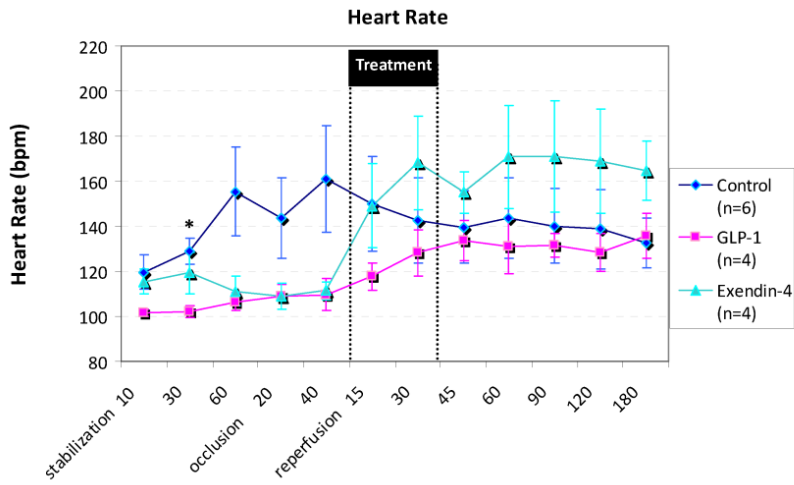
Hemodynamics

The hemodynamic plotted in Figure A.13. Data include heart rates, mean arterial blood pressures (ABP), systolic ABP, diastolic ABP, cardiac output (CO), left ventricular

systolic pressure (LVSP), left ventricular end diastolic pressure (LVEDP), ±LV dP/dt, and LV Tau. Systolic and diastolic arterial pressures were not available for the control group, but comparisons were made between treatment groups.

Of the parameters assessed, only mean and diastolic arterial pressures showed significant differences during the infusion period, with the Exendin-4 group higher compared to the GLP-1 group only (P<0.05). LV –dP/dt as well as LV systolic and developed pressures showed differences between groups prior to infusions as well as during infusions. The heart rate did increase in the infusion period in the GLP-1 and Exendin-4 groups by approximately 20 to 50 bpm, respectively, but the increase was not statistically significant. In both treatment groups, the rise in heart rate plateaued 30 minutes into reperfusion which coincided with cessation of infusions in dosing regimens A and B employed in 7 of 8 animals.
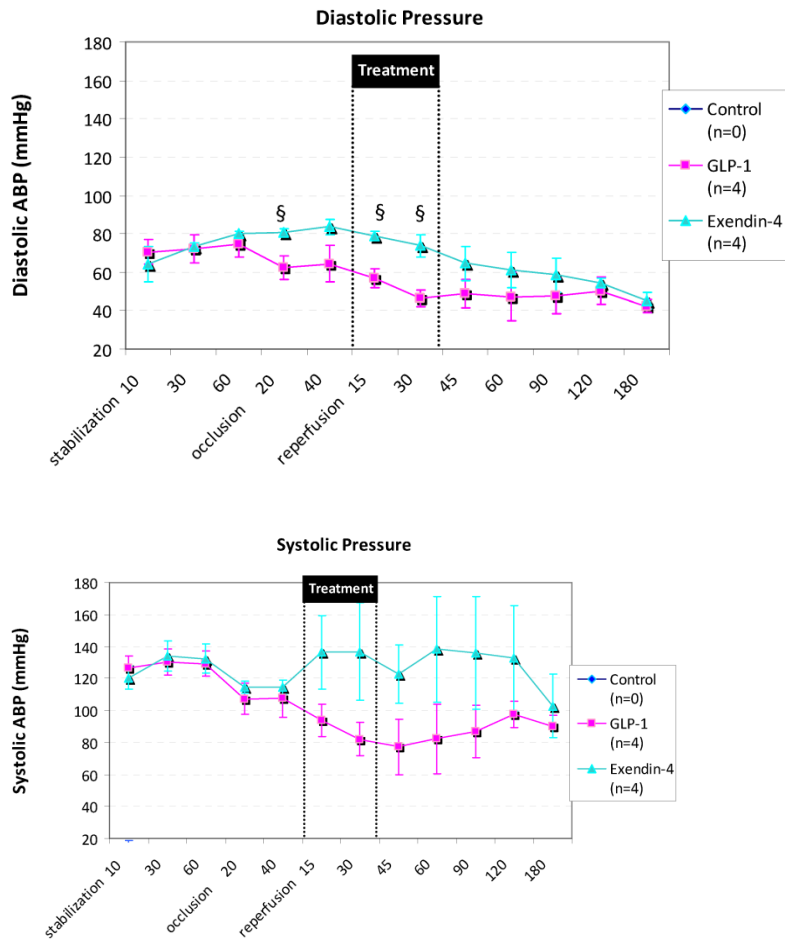
Heart Rate

* P<0.05 GLP-1 vs. Control
† P<0.05 Exendin-4 vs. Control
§ P<0.05 GLP-1 vs. Exendin-4

Mean Arterial Blood Pressure
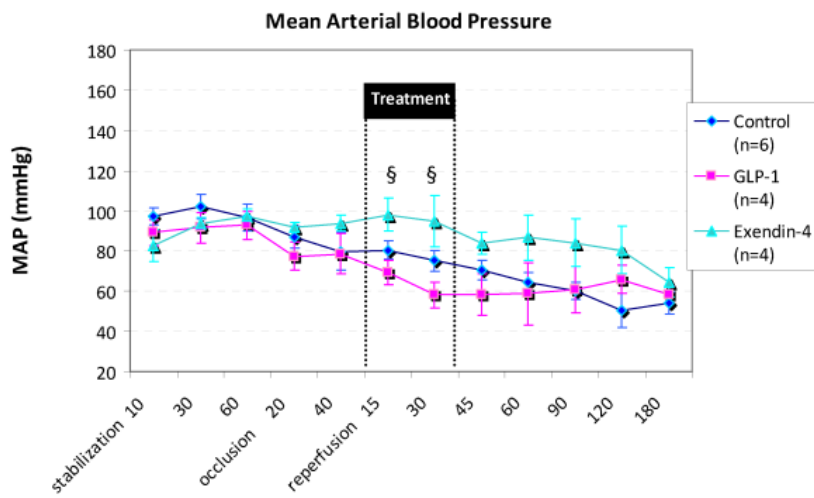
Figure A.13: Hemodynamic parameters including heart rate, mean arterial pressure, arterial diastolic pressure and arterial systolic pressure. Mean arterial and diastolic pressures in Exendin-4 animals are significantly higher compared to GLP-1 treated animals

Pharmacokinetics of GLP-1

The total detected peptide is expressed as the area under the curve (AUC), which is displayed in Table A.4 along with maximum concentration (C_{max}) achieved for each animal, as well as infarct area. Area under the curve is higher in Animal #8 compared to others in the same treatment group due to the dosing regimens employed. Of particular interest is that, though infusion rate was increased from 1.5 to 2.5 pmol/kg/min in Animal

8, the C_{max} is not reflective of the higher dose. In the Exendin-4 group, all animals received the same total dose under two dosing regimens administered over 30 minutes; however, the Exemdom-4 bolus loading dose in Animals 6 and 7 did not afford a comparable C_{max} and AUC in relation to continuous infusion. Representative traces of detected peptides are shown in Figure A.14.

Group	Animal #	AUC (pM·h)	Cmax (pM)	Infarct Area (% risk zone)
GLP-1(7-36)	1	18.5	43.2	48.5%
	2	5.1	12.7	43.3%
	3	15.7	36.4	52.1%
	8	55	25.5	77.6%
	Mean ± SD	23.6 ± 21.7	29.5 ± 13.3	55.4 ± 15.2%
Exendin-4	4	192.6	233.4	2.79%
	5	213.6	297.1	18.13%
	6	110.4	97.8	33.01%
	7	30.7	77	19.41%
	Mean ± SD	136.8 ± 83.6	176.3 ± 106.3	18.3 ± 12.4%

Table A.4:   Area under the curve (AUC), maximum concentration (Cmax). and Infarct area for GLP-1 and Exendin-4

#4
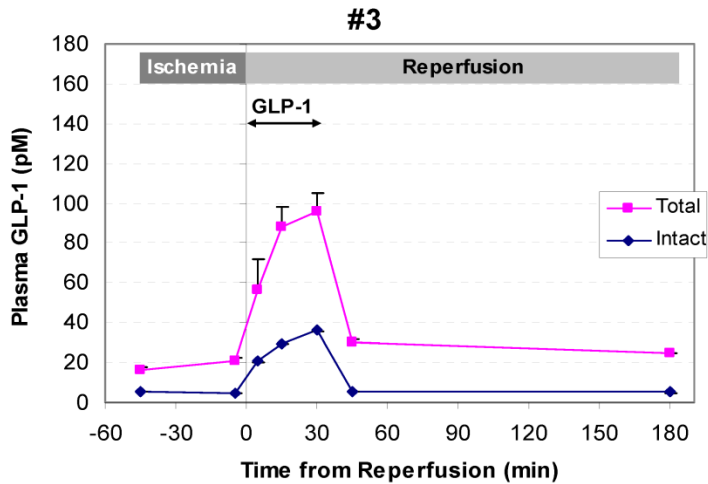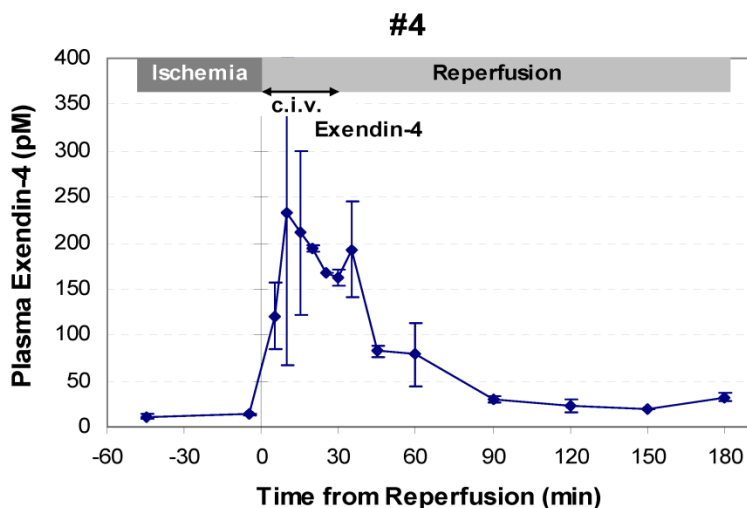
Figure A.14: Representative traces of plasma levels of intact (GLP-1(7-36)) and total GLP-1 (GLP-1(7-36) + GLP-1(9-36)) in pig #3 (top) and plasma levels of Exendin-4 in pig #4. Ischemia and reperfusion periods are displayed at the top, as well as the 30 minute infusion period for the treatment.

Metabolics

Insulin levels were measured in 3 animals (2 GLP-1 treated) as well as myocardial glucose balance in all animals. Measurable increases in insulin were detected during treatment periods with both GLP-1 and exendin-4 confirming a physiologic response was elicited through peptide delivery. Glucose levels in several of the animals were slightly elevated, which is contrary to the expected response (data not shown).

Discussion

Two GLP-1 analogs were investigated in this study to evaluate the cardioprotective potential of these peptides. At the time the study was initiated, there was significant preclinical evidence in rodents, though some of it was controversial with conflicting results, and only a small number of studies in higher species.

In vitro model

After four hours of functioning *in vitro*, the peak forces produced by the non-hypoxic trabeculae dropped to 70-85% of initial values. Neither dose level of GLP-1 nor Exendin-4 affected contractile function in this model. This suggests that neither has inotropic effect in porcine myocardial tissue.

We saw that subjecting trabeculae to hypoxia and reoxygenation inflicts injury such that they can only recover to 50-60% of their original force after three hours of reperfusion. This is lower than trabeculae not subjected to hypoxia, indicating the hypoxic period damaged the myocardium. However, treatment of swine myocardial tissue with GLP-1 or exendin-4 at 3nM or 0.3nM concentrations had no observable effects that were manifested in this model. This indicates that any contractile effects may be due to systemic effects of a given treatment, rather than direct action on the tissue.

Acute MI model

Dosing of 1.5 pmol/kg/min and duration of infusion of 30 minutes initiated just prior to infusion was chosen from the literature [5], [27–29]. As the study was being conducted, the Timmers study was published showing efficacy with a slightly different dosing strategy of Exendin-4 in which one IV dose and one subcutaneous dose were administered simultaneously 5 minutes prior to reperfusion [9]. Their dosing strategy was adopted in this study (dosing scheme B) with a bolus of Exendin-4 preceding continuous IV infusion for 30 minutes to emulate the slower absorption and bioavailability of the subcutaneously delivered peptide in the Timmers study. While some reduction in infarct size was observed in the Exendin group, little response was

observed in the GLP-1 group, corroborating the study by Kavianipour reporting no reduction in infarct size or improvement in hemodynamics with GLP-1 [16]. In the final animal, the dose was increased and the infusion duration extended in response to input from a physician consultant. Surprisingly, the infarct size was larger in this high-dose experiment compared to others in the same group or in the control group. There has been one similar report in the literature with low-dose Exendin-4 reducing infarct area while high dose was ineffective [18]. Further replicates would be needed to determine if the higher dose is disadvantageous or less effective in cardioprotection.

Despite the limitations of this small investigational study discussed herein, the key finding remains that Exendin-4, and not GLP-1(7-36), was shown to reduce infarct size. While some similar studies in isolated rodent hearts have shown GLP-1(7-36) to be cardioprotective [4], [5], [8], other studies have shown cardioprotection in either intact or isolated rodent hearts with GLP-1 only when it was co-administered with a DPP-4 inhibitor [3], [7], or with exendin-4 [18] or other DPP-4-resistant analogs [30]. Literature in higher species is limited. Within swine, our results on reduction of infarct area are consistent with the two studies in the literature [9], [16]. Specifically, Timmers et al. [9] reported a ~40% reduction with delivery of Exendin-4 compared to controls (32.7 ±6.4% vs. 53.6 ± 3.9%; p=0.031); the present study shows a 38% reduction in the Exendin-4 group (13.3 ± 12.4% vs. 48.7 ± 19.7%, p=0.03). There is notably more variation in our study compared to Timmers which highlights the importance of conducting additional studies to confirm this difference. With infusion of GLP-1(7-36), an earlier study showed only improved metabolics (decreased lactate and glucose levels but increased insulin) without reduction in infarct size or improved hemodynamics compared to controls [16].

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The presence of GLP-1 and exendin-4 in their respective animals confirmed successful delivery. Likewise, the present study showed no improvement in infarct size or hemodynamics in the GLP-1(7-36) group. The cardioprotective effects of GLP-1 and its analogs in acute ischemia may be species specific.

While some hemodynamic responses were observed, the impact on cardiac function was not conclusive in this study. It is possible that myocardial stunning in this model may prevent any potential acute benefit regardless of infarct size, and that longer recovery over several hours or days may elucidate the beneficial response from the intervention, such as seen in the Timmers study. However, the observed increase in mean and diastolic ABP in the exendin-4 group may have improved myocardial perfusion and aided in reducing infarct size.

The literature is conflicting over whether or not GLP-1 and analogs have an effect on heart rate. Due to the surgical intervention required in these experiments, the ischemia-reperfusion model may not be the ideal experimental conditions to test the effect on heart rate. However, there was a marked, though not significant, rise in heart rate during infusions in both treatment groups that was not observed in the control group.

Limitations

The major limitations of this study are inadequate sample size and use of historical controls with slight protocol variations from the current protocol. Additional studies would increase confidence in the results, but were planned, but the limited available

resources were reprioritized to focus on chronic preclinical studies in existing heart failure models. Use of historical controls prohibited improvements in the study protocol; for example, some of the data of interest in this study, such as myocardial glucose balance and insulin plasma levels, were not available from the prior study. Since GLP-1 is not being proposed as an antiarrhythmic, administration of amiodarone or other antiarrhythmics to prevent VF episodes would allow for a better assessment of metabolics and cardioprotective effects of GLP-1 without the added confounding factor. A further improvement would include recovery for at least two days prior to sacrifice. Additionally, there is some speculation that GLP-1 activates two pathways: one being the cAMP/Akt pathway for acute effects, while a secondary pathway requires at least 24 hours of continuous infusion to elicit the response. The Timmers study [9] employed this model and allowed reperfusion for three days with treatment with Exenatide continuing for two days resulting in a significant reduction in infarct size and loss of cardiac function.

Conclusions

Intravenous administration of Exendin-4, but not GLP-1(7-36) amide, significantly reduced infarct size in this small investigational study. Though there was no significant difference in arrhythmia score among treatment and control groups, one or more episodes of ventricular fibrillation occurred in 50% of the Exendin-4 group, 25% of the control group, and 0% of the GLP-1 group. There were no mortalities in either treatment group, while the control group experienced 17% mortality [23]. The effect of GLP-1 or analogs on hemodynamics and cardiac function post-ischemia is not clear in this study. GLP-1 or Exendin-4 was detected in the plasma of the respective treatment groups, confirming efficacious delivery of the peptides. Plasma insulin increased during

treatment period in 2 of the 3 animals tested, one in the GLP-1 group and one the

Exendin-4 group, which may be due to administration of these insulinotropic peptides.

The lack of effect on isolated trabeculae suggests any improvement in hemodynamics

may be due to systemic effects of the treatment.

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