Role of furan metabolites in cell toxicity and carcinogenesis

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Abstract

Furan is a product of incomplete combustion present in canned foods, tobacco smoke and exhaust fumes. It has been shown to be a liver toxicant and carcinogen in rodents. This project seeks to determine the toxic carcinogenic effect of furan in human liver cells as these remains unknown. *Cis*-2-butene-1,4-dial (BDA), a highly reactive breakdown product of furan, has been shown to form stable crosslinks with polyamines and glutathione *in vivo* and in cell models. The spermine and ornithine crosslinks were synthesized and purified using high pressure liquid chromatography and characterized using mass spectrometry and nuclear magnetic resonance. The synthesized crosslinks will be introduced into human liver cell cultures to study if these products of furan metabolism play a role in liver toxicity and carcinogenicity. These data will be combined with others to determine if furan is a human health concern.

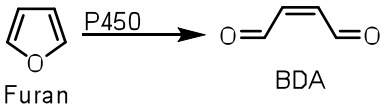
Introduction

•Furan is a product of incomplete combustion found in canned and jarred foods as well as in smoke (tobacco, wood and exhaust). It has been shown to cause liver toxicity and cancer in rats and mice.

•Furan is classified as a possible human carcinogen by IARC since the risk of exposure to it is high. For furan to exert its toxic effects however, it needs to be metabolized.

•This project sought to synthesize, purify and characterize the crosslinks formed with polyamines by one such furan metabolite, to determine if these crosslinks are linked to furan toxicity.

•Furan is oxidized to *cis*-2-butene-1, 4-dial (BDA) by microsomal enzymes. This reactive dialdehyde readily reacts with glutathione (GSH) nonenzymatically to form conjugates.



Polyamines and Cell Function

•Polyamines are organic compounds present in cells that have multiple amine groups.

•Their specific function is unknown but they are thought to play a role in cell growth as their absence leads to growth retardation or inhibition.

•It is our theory that the formation of these BDA-polyamine crosslinks is a cause of cell toxicity.

Introduction of Project

•Goal: To synthesize, purify and characterize compounds that contain known metabolite of furan (BDA) and test them for toxicity.

•Study Design: Glutathione monoethylester (GSH-EE) was reacted with polyamines and BDA to yield cross-links that can be used to study cancer and toxicity pathways.

Synthesis and purification:

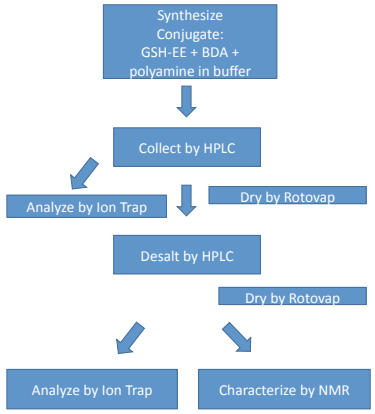
– High Performance Liquid Chromatography (HPLC) with UV detection was highly employed using a Synergi HPLC column.

–GSH-EE was used in the syntheses instead of GSH, since the ester group allows compounds to easily cross cell membranes

Characterization:

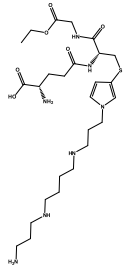
–Mass spectrometry was performed on an MSD Ion Trap for characterization.

Methods



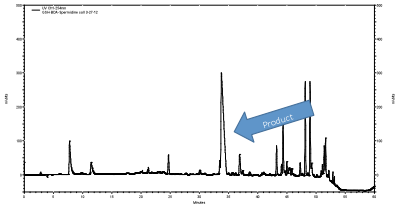
Synthesis of Polyamine Conjugates

•The desired polyamine was dissolved in sodium phosphate buffer.
•GSH-EE was added to this and thoroughly mixed.
•BDA was added last and the reaction mixture let to sit for an hour to ensure maximum crosslink yield.
•The concentrations of the reagents were all the same at 25 mM.
•The solution was then run on the HPLC, 100 µl at a time to collect peaks with the desired product.



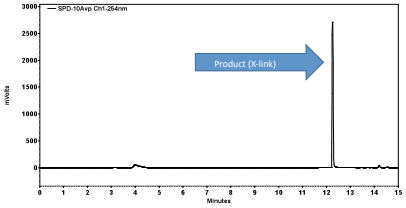
Results/Data

The GSH-EE-BDA-polyamine conjugates were collected using a method with 50 mM ammonium formate buffer, pH 2.8, and 50% acetonitrile.



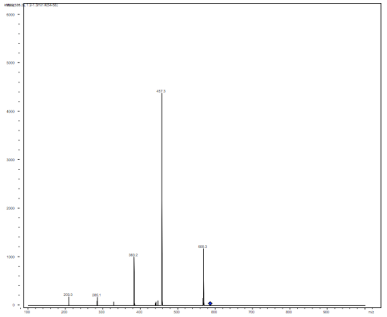
Desalting

The samples collected were desalted using a HPLC method with 0.1% formic acid and 100% acetonitrile in order to remove excess buffer salts. The remaining solution was dried further on the rotavaporator.



Characterization: Mass Spectrometry

•All collected peaks were analyzed for their fragmentation spectra using an MSD Ion Trap.
•This was done to ensure that our collected peaks contained the desired crosslink before proceeding further.



Conclusion

•Synthesis of GSH-BDA-polyamine conjugates was shown successful as evidenced by ion trap analysis.
- Mass spec analysis of the collected peaks showed desired products at 586 *m/z* (spermine) and 516 *m/z* (ornithine).
- A conjugate with spermidine has been synthesized but still needs to be characterized.
•The next step from here will be to further characterize the crosslinks using NMR technology.

Future Directions

•The crosslinks will be introduced into human liver cell cultures to observe their toxic and/or carcinogenic effects.
•This new information will be combined with existing data in order to determine if furan is a human health hazard and to make recommendations to reduce our exposure to it.

Acknowledgements

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