Protection against Acute Kidney Injury by TUDCA

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Abstract

Presently there is no therapy for acute kidney injury (AKI). Potentially one can protect kidneys against injury by preventing cell death following AKI. There are two types of cell death that occurs following AKI: necrosis and apoptosis. Necrosis is an uncontrolled and synchronous cell death that occurs at the time of AKI. In contrast, apoptosis, or programmed cell death is an asynchronous cell death that continues to occur for days following AKI, thereby providing a window of opportunity for intervention. Tauroursodeoxycholic acid (TUDCA), a bile acid synthesized in the liver, has been shown to be effective by inhibiting apoptosis in rat models of stroke and Huntington’s disease. We hypothesized that TUDCA will be similarly effective in protection against AKI. Accordingly, the goal of this study was to investigate the protective effects of TUDCA in a rat model of ischemic AKI. We induced AKI by bilateral renal artery clamping for 45 minutes. Three rats were given 400mg/kg/day of TUDCA intra peritoneally from day-1 to day 6, while the control animals received the vehicle. We determined kidney functions by measuring blood urea nitrogen (BUN), proliferation by immunohistochemistry for Ki-67, and apoptosis by TUNEL assay.

In conclusion, TUDCA provides protection against acute kidney injury likely by preventing apoptosis.

Methods

Rat Model of Acute Kidney Injury: Acute kidney injury was induced by bilateral renal artery clamping using vascular clamps for 45 minutes at 37 degree C.

Dosing Regimen of TUDCA: TUDCA was given intraperitoneally in the dose of 400mg/kg/day in Phosphate buffered saline (PBS) to the experimental group from day-1 to day 6. Control animals received equal volume of PBS.

Measurement of Kidney Functions: Kidney functions were determined by measuring blood urea nitrogen in the serum levels by Colorimetric method. (Fig. 1a and 1b).

Determination of Proliferation and Apoptosis: We determined proliferation by immunohistochemistry for Ki-67 and apoptosis by TUNEL assay in formalin fixed paraffin embedded sections from regenerating kidneys.

• TUNEL Assay: We determined apoptotic cells using TUNEL assay as published protocol (Millipore) using apoptosis detection kit 15302 (Cat# 17-141). In brief, this method involved identification of positive nuclear stains by using a terminal deoxynucleotidyl transferase (TdT) to transfer biotin-dUTP to the free 3’-OH of cleaved DNA. The biotin-labeled cleavage sites are then visualized by reaction with peroxidase conjugated avidin. (Fig. 2a and 2b).

• Immunohistochemistry: The protocol involved overnight incubation at 4 degree C of kidney section with 1:25 dilution of anti-Ki-67 primary antibody (Thermo Scientific Cat # RM-9106) followed by peroxidase labeled secondary antibody (anti-rabbit/mouse IgG) for 30 minutes. 3,3-Diaminobenzidine (DAB) was used as the peroxidase substrate for color development. (Fig. 3a and 3b).

Results

Effect on Kidney Function

Figure 1a: Effect on Kidney Function

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Figure 1b: This figure represents comparison of peak BUN levels post injury (%) in experimental and control groups (165mg/dl vs. 130mg/dl).

Significance: TUDCA significantly attenuates peak injury following ischemic AKI.

Figure 2a: Representative image of TUNEL assay performed on kidney section from control (2a) and experimental animals (2b). Apoptotic cells are identified as DAB positive brown nuclei (arrow). The sections were counter-stained with hematoxylin. Total number of DAB positive apoptotic cells in the entire kidney section were counted using Spectrum WinViewer software (2a). There were 219 DAB positive cells in the control group compared to 132 in the experimental group.

Significance: TUDCA protects kidney likely by anti-apoptotic mechanism.

Conclusions

TUDCA is effective in protecting against ischemic acute kidney injury likely by an anti-apoptotic mechanism.

Future Directions

• Validation in a larger sample size
• Validation in other models of acute kidney injury
• Optimization of TUDCA dosing regimen
• Determination of target molecules of the apoptosis pathway

References

