RENOPROTECTION WITH PITUITARY ADENYLATE CYCLASE-ACTIVATING POLYPEPTIDE IN CYCLOSPORINE A-INDUCED NEPHROTOXICITY.

Altaf-M Khan*, Min Li*, Elizabeth Brant*, Kristine E. Gullo*, Wei Cai*, Jerome L. Mederdu***, Eric E Simon*, and Vecihi Batuman*

*Section of Nephrology and Hypertension, and **Peptide Research Laboratory, Department of Medicine, Tulane University School of Medicine, New Orleans, LA.

Cyclosporine A (CsA) is a widely used immunosuppressant drug for post-allogeneic organ transplantation. The major limiting factors for using CsA are its acute and long-term toxic effects on the kidney, resulting in glomerular sclerosis, tubular atrophy and interstitial fibrosis. Our recent studies have shown that pituitary adenylate cyclase-activating polypeptide (PACAP) protects the kidney from ischemic and nephrotoxin-induced renal injury both in vitro and in vivo by modulating immune responses. This study evaluates the effects of PACAP38 in CsA-induced nephrotoxicity in human renal proximal tubule epithelial (HK-2) cells and in mice exposed to CsA. Fifty µM CsA caused marked cellular morphological alterations in HK-2 cell cultures, accompanied by increased expression of the profibrotic cytokine TGF-β1. The addition of 10⁻⁸ M PACAP38 triggered the formation of cell aggregates and reduced TGF-β1 secretion and CsA-induced cytotoxicity as indicated by ELISA and LDH assays, respectively. For the in vivo studies, male BALB/c mice (8-10 weeks-old) were given a single intraperitoneal injection of CsA (5 mg/kg b.w.). The treatment groups received 20 µg of PACAP38 2 hr before the exposure to CsA and additional doses were given every day for ten days. CsA exposure caused severe tubular injury characterized by the extensive loss of tubular epithelial cells and brush border membranes, tubular collapse, and cellular necrosis and interstitial fibrosis. Exposure to CsA significantly stimulated the production of the fibrogenic cytokine TGF-β1 in mouse kidneys. The treatment with PACAP38 resulted in a decrease in kidney TGF-β1 and TNF-α production, and in serum creatinine levels. PACAP38 significantly reduced renal tubular injury, inhibited the profibrotic cascade by suppressing vimentin, fibronectin, laminin, and octamer 4 expression by immunohistochemistry, and blocked epithelial-mesenchymal transition (EMT) of the renal cells as shown by the restoration of E-cadherin and ZO-1 and the suppression of CsA-induced α-SMA and E2A expression in CsA-exposed mice by Western blot analysis. Moreover, PACAP38 attenuated the expression of MCP-1, IL-6 and TNF-α, and restored the expression of collagen IV and CXCL1 in CsA-exposed murine kidneys as analyzed by real-time RT-PCR. Interestingly, PACAP38 also markedly suppressed the production of reactive oxygen species (ROS) levels by NADPH in the kidney cortex of mice exposed to CsA. We have demonstrated that the exposure to a clinically relevant dose of CsA provoked EMT in mouse kidneys. PACAP38 effectively suppressed the EMT process, and TGF-β1 and ROS production to prevent tubulointerstitial fibrosis and cell injury both in vitro and in vivo. This effect could be due to the action of PACAP38 on ROS via its inhibition of phagocytic NADPH oxidase (NOX2). PACAP could be developed as a novel renoprotective agent for CsA-induced nephrotoxicity.

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