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Glutamine Donor Pretreatment in Rat Kidney Transplants with Severe Preservation Reperfusion Injury[1]

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Background. **Glutamine (GLN) has been shown to confer cytoprotection by enhancing endogenous heat shock protein (HSP) expression. We hypothesized that GLN donor pretreatment protects rat renal grafts against severe preservation reperfusion injury (PRI).**

Materials and methods. **GLN (0.75 g/kg) or saline was administered i.p. to male donor rats 24 h and 6 h before donor nephrectomy. Kidneys (n = 6/group) were cold-stored in UW solution for 40 h and transplanted into bilaterally nephrectomized syngeneic recipients. Grafts were removed after 24 h. Renal HSP 70 expression was determined by Western blotting. Graft function was assessed by serum creatinine. Renal cross sections were microscopically examined for acute tubular necrosis, apoptosis, tubular proliferation, and macrophage infiltration.**

Results. **GLN donor pretreatment significantly increased intragraft HSP 70 expression. Serum creatinine was not different between groups: 2.6 ± 0.2 mg/dL (saline) *versus* 2.7 ± 0.5 mg/dL (GLN). Both treatment groups showed severe tubular damage with significantly less papillary necrosis in the GLN group (P < 0.05). GLN significantly reduced the number of apoptotic tubular cells in the cortex, medulla, and papilla (P < 0.001 *versus* saline). Postinjury tubular proliferation, measured by PCNA antigen expression, and intragraft macrophage infiltration was not influenced by GLN.**

Conclusions. **In rat renal grafts suffering severe PRI pharmacological preconditioning with GLN attenuates early structural damage, especially tubular cell apoptosis. Stimulation of renal HSP 70 expression could be an important mechanism of GLN-induced cytoprotection. Our findings may have implications for the treatment of delayed graft function in recipients of marginal donor kidneys.**

Key Words: **kidney transplantation; rat; ischemic injury; glutamine; heat shock protein; apoptosis.**

INTRODUCTION

Kidneys from marginal donors have an increased incidence of delayed graft function (DGF). Clinically applicable donor pretreatment protocols to treat DGF in recipients of marginal renal grafts are not well established. We used a syngeneic rat kidney transplantation model with severe preservation reperfusion injury (PRI), induced by 40 h of cold storage, to test potential benefits of glutamine donor pretreatment.

Glutamine (GLN), an amino acid conditionally essential during critical illness and injury, has been shown to reduce cell and organ damage induced by endotoxemia or ischemia [1, 2]. As previously shown, glutamine induces endogenous heat shock protein 70 (HSP 70) expression in animals and humans, thus conferring cytoprotection against various stressors [3, 4]. Glutamine has been reported to improve outcomes in experimental models of intestinal and cardiac ischemia reperfusion injury (IRI) [2, 5]. In animal models of renal IRI, elevated HSP 70 levels have been associated with improved functional outcomes [6, 7].

In our present study, we hypothesized that glutamine donor pretreatment, initiated 24 h before organ

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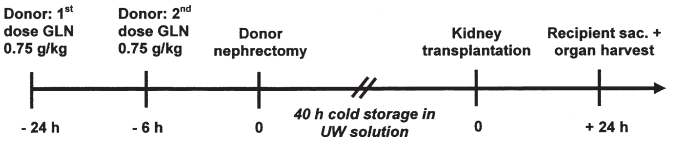
procurement, induces renal HSP 70 expression and attenuates early structural damage and functional impairment in 40 h cold-preserved syngeneic rat kidney grafts.

MATERIALS AND METHODS

GLN Administration and Experimental Design

GLN was administered as an alanyl-glutamine dipeptide (Fresenius-Kabi, Homburg, Germany), which was dissolved in saline. GLN solutions were filtered with a 0.45-μm filter before intraperitoneal administration. Donor rats received either 0.75 g/kg GLN or saline (n = 6 per group) 24 h and 6 h before left donor nephrectomy. Grafts were cold-stored in UW solution for 40 h and transplanted into bilaterally nephrectomized syngeneic recipients. In the sham operation group, animals (n = 3) underwent right nephrectomy and the contralateral kidney was used for additional experiments. Animals were sacrificed 24 h after surgery for blood sampling and organ harvesting. Kidneys were cross-sectioned and stored in preparation for Western blot analysis, conventional histology, and immunohistochemistry.



Animal Surgery

All animal protocols were reviewed and approved by the University of California San Francisco Committee on Animal Research, and animal care was in agreement with the National Institutes of Health (NIH) guidelines for ethical animal research (NIH publication number 80-123, revised in 1985). Kidney donors and recipients were inbred male Lewis rats (200 to 250 g; Charles River Laboratories, Wilmington, MA) housed under standard conditions with free access to water and chow. All procedures were performed under inhalation anesthesia with isoflurane. Left kidneys were procured, flushed with ViaSpan, University of Wisconsin (UW) solution and stored at 4°C for 40 h.

Recipients underwent bilateral native nephrectomies followed by heterotopic kidney transplantation using an established microsurgical technique [8]. Briefly, end-to-side anastomoses between the renal vessels and the recipient's abdominal aorta and inferior vena cava were created using continuous 8-0 nylon sutures; mean warm ischemia time was 17 ± 1.2 min. An end-to-end ureteroureterostomy was performed using interrupted 11-0 nylon suture. After recovery from anesthesia, animals were transferred to the housing facility and monitored until sacrifice 24 h post-transplantation.

Heat Shock Protein Detection

Renal cross sections including cortex, medulla, and papilla were snap frozen in liquid nitrogen and stored at −80°C until analysis. Western blotting was performed as previously described [1]. Blots were blocked with a 5% milk PBS Blotto solution. For HSP 70 detection, blots were incubated with a primary mouse-anti-HSP 70 antibody (Stressgen, Victoria, Canada). Blots were washed and incubated with a horseradish peroxidase-conjugated secondary goat-anti-mouse antibody (Santa Cruz Biotechnology, Santa Cruz, CA). Densitometry was determined using the UVP Chemiluminescent Darkroom System (UVP Inc., Upland, CA).

Graft Function

Graft function was assessed by serum creatinine measurement 24 h post-transplantation. Recipient blood samples (0.5 mL) were processed by IDDEX Veterinary Services (Sacramento, CA).

Tubular Necrosis Score

For morphological studies, formalin-fixed renal grafts (n = 6 per group) were used. Paraffin cross sections (4 μm) were stained with hematoxylin and eosin and periodic acid-Schiff (PAS) using standard procedures. A renal pathologist, blinded for the experimental conditions, examined and scored kidney sections for acute tubular necrosis (ATN) as previously described [9]. ATN was graded as follows: 0 represented no abnormalities, and 1, 2, 3, and 4 represented slight (<20%), moderate (20 to 40%), severe (40 to 60%), and near-total (>60%) necrosis of the renal parenchyma, respectively.

Apoptosis Detection

Renal apoptosis was examined using the in situ Cell Death Detection Kit (Roche Diagnostics, Indianapolis, IN) based on the TdT-mediated dUTP nick end labeling (TUNEL) method. Paraffin-embedded renal cross sections including the cortex, medulla, and papilla were treated according to the manufacturer's instructions and stained with Fluorescein. TUNEL-positive apoptotic cells were visualized under a fluorescence microscope (Zeiss Axio Imager A1, Jena, Germany) and quantitated using a digital imaging system (Zeiss Axiocam HR with Axiovision 4.4 software). In each microcompartment, 10 to 15 randomly chosen fields of view (FOV) per section were evaluated. The means of six samples were grouped together to obtain a final mean ± SD. Positive cell staining was recorded digitally and expressed as the percentage of TUNEL-positive area per FOV at 400× magnification.

Immunohistochemistry

For assessment of intragraft monocyte/macrophage infiltration a monoclonal mouse anti-ED1 antibody (endothelial 1 antigen on monocytes/macrophages; Serotec, Oxford, United Kingdom) was used. For assessment of tubular cell proliferation a monoclonal mouse anti-PCNA (proliferating cell nuclear antigen, clone PC10; Zymed Laboratories Inc., San Francisco, CA) was used. Immunohistochemistry was carried out on 2 μm paraffin sections. ED1+ staining was performed using the alkaline-phosphatase-anti-alkaline-phosphatase (APAAP) method on paraffin sections after deparaffinization and rehydration. Sections were incubated for 60 min at room temperature with primary Ab at 1:1000 dilution in RPMI (Seromed, Heidelberg, Germany) with 10% fetal calf serum (FCS) and 3% bovine serum albumin (BSA). Fast Red Kit (DakoCytomation, Hamburg, Germany) was used for detection and visualization.

PCNA staining was performed using the standard avidin-biotin-complex method (Dako). Sections were deparaffinized in xylol, rehydrated through graded alcohols and cooked in citrate buffer (pH 6.0) for 5 min. Slides were then incubated with primary Ab (diluted at 1:100) for 60 min at room temperature in Ab-diluent (Dako). AEC-chromogen (Dako) was used for visualization. Sections incubated with corresponding isotype controls instead of primary Ab were used as negative controls.

Intragraft ED1 and PCNA expression were evaluated in a blinded fashion. In the cortex, medulla, and papilla, 10 to 15 randomly chosen FOVs per section were evaluated using a light microscope (Zeiss Axio Imager A1, Jena, Germany). Positive cell staining was expressed as mean ± SD of cells per field of view (FOV).

Statistical Analysis

Values are expressed as means ± SD. If not otherwise indicated, groups were compared with the unpaired Student's t-test. For com-