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Aging aggravates long-term renal ischemia-reperfusion injury in a rat model

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ARTICLE INFO

Article history:
Received 25 February 2013
Received in revised form
26 July 2013
Accepted 4 October 2013
Available online 10 October 2013

Keywords:
Ischemia/reperfusion
Transforming growth factor beta 1
Oxidative stress
Aged rats
Reactive oxygen species
Malondialdehyde
8-Hydroxydeoxyguanosine

ABSTRACT

Aim: Ischemia-reperfusion injury (IRI) has been considered as the major cause of acute kidney injury and can result in poor long-term graft function. Functional recovery after IRI is impaired in the elderly. In the present study, we aimed to compare kidney morphology, function, oxidative stress, inflammation, and development of renal fibrosis in young and aged rats after renal IRI.

Materials and methods: Rat models of warm renal IRI were established by clamping left pedicles for 45 min after right nephrectomy, then the clamp was removed, and kidneys were reperfused for up to 12 wk. Biochemical and histologic renal damage were assessed at 12 wk after reperfusion. The immunohistochemical staining of monocyte macrophage antigen-1 (ED-1) and transforming growth factor beta 1 (TGF-β1) and messenger RNA level of TGF-β1 in the kidney were analyzed.

Results: Renal IRI caused significant increases of malondialdehyde and 8-hydroxydeoxyguanosine levels and a decrease of superoxide dismutase activity in young and aged IRI rats; however, these changes were more obvious in the aged rats. IRI resulted in severe inflammation and tubulointerstitial fibrosis with decreased creatinine (Cr) clearance and increased histologic damage in aged rats compared with young rats. Moreover, we measured the ratio of Cr clearance between young and aged IRI rats. It demonstrated that aged IRI rats did have poor Cr clearance compared with the young IRI rats. ED-1 and TGF-β1 expression levels in the kidney were significantly higher in aged rats than in young rats after IRI.

Conclusion: Aged rats are more susceptible to IRI-induced renal failure, which may associate with the increased oxidative stress, increased histologic damage, and increased inflammation and tubulointerstitial fibrosis. Targeting oxidative stress and inflammatory response should improve the kidney recovery after IRI.

1. Introduction

The aging process is a multifactorial biological phenomenon, which is characterized by the loss of adaptive responses to physiological stress, resulting in an increased susceptibility to disease and death [1]. There is considerable evidence that aging occurs as a consequence of oxidative stress, and reactive oxygen species (ROS) produced during cellular

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http://dx.doi.org/10.1016/j.jss.2013.10.008

metabolism leads to an age-dependent increase in oxidatively modified proteins, lipids, and nucleic acids in tissue. ROS are crucially involved in the pathophysiology of ischemia-reperfusion injury (IRI). However, the influence of age on organ susceptibility to the IRI in a rat model of aging has not been thoroughly studied.

The growing organ shortage represents the largest problem in organ transplantation. As a consequence, increased numbers of so-called "marginal" or extended criteria donor grafts are used. Although the term marginal donor remains ill-defined, donor age seems to be a critical factor for the quality of the graft. In clinical practices, we have the feeling that aged people donated kidney had slow functional recovery after kidney transplantation. More and more experimental and clinical studies have demonstrated the influential role of donor and recipient age for the development of chronic graft dysfunction [2–6]. Moreover, much attention has been focused on events occurring during an ischemic event or in the early recovery phase, but the long-term effects of ischemic injury remain unclear. In this study, we aimed to perform long-term follow-up of the changes in renal function and structure in young and aged rats after their exposure to IRI and investigate the mechanism responsible for these changes.

2. Materials and methods

2.1. Animals

Three-month-old (young) or 24-month-old (aged) Sprague-Dawley rats were used, approved by the Institutional Animal Care and Use Committee. All experiments were performed in accordance with the National Institute of Health guidelines (NIH Publ. No. 86-23, revised 1985). Before the experiments, the animals were fed a standard rat chow, drank water *ad libitum*, and were housed in metabolic cages under controlled temperature in 12-h light/dark cycles for at least 1 wk. The rats were randomly assigned to four groups ($n = 10$): (1) young rats that underwent right nephrectomy (young sham); (2) aged rats that underwent right nephrectomy (aged sham); (3) young rats that underwent ischemia-reperfusion injury (young IRI); (4) aged rats that underwent ischemia-reperfusion injury (aged IRI). Rat models of warm renal ischemia-reperfusion were established by clamping left pedicles for 45 min after right nephrectomy, then the clamp was removed, and kidneys were reperfused for up to 12 wk. Twelve weeks after the reperfusion, all rats were killed under 100 mg/kg pentobarbital anesthesia, and their kidney tissues were removed.

2.2. Surgical procedures

We followed a surgical procedure described in previous study [7], all procedures were performed aseptically. The animals were anesthetized with a combination of ketamine hydrochloride (85 mg/kg) and xylazine hydrochloride (15 mg/kg) and placed on a heating pad. Laparotomy was performed through a midline incision. Then, the right renal artery and right ureter were ligated and right nephrectomy was performed. The left renal pedicle was exposed and occluded with a nontraumatic vascular clamp for 45 min (except for the young and aged sham group) and left kidneys were then reperfused for up to 12 wk. Occlusion was confirmed by a significantly pallid change of the kidney color and a return to a red shade on reperfusion. Rats were killed under anesthesia, blood was drawn, and the left kidneys were harvested and frozen in liquid nitrogen.

2.3. Analysis of renal function

Renal function in 12 wk post-ischemic kidneys was assessed by measuring serum blood urea nitrogen (BUN) and creatinine (Cr) levels. The samples were analyzed on a COBAS Mira chemical analyzer (Roche, Basel, Switzerland) with commercial kits from Sigma (St Louis, MO).

2.4. Cr clearance

Urine was collected for 24 h in the last day of 12 wk post-ischemic kidneys in metabolic cages. Serum and urine Cr levels were determined using standard assays (Sigma creatinine kit). Urine volume was determined gravimetrically. Cr clearance over 24 h was calculated as $(U_{creatinine} \times V)/P_{creatinine}$, where $U_{creatinine}$ and $P_{creatinine}$ were urinary and plasma Cr levels, respectively, and V was urine volume per minute. Urine volume per minute was calculated as the total of urine volume divided by 720 min.

2.5. Histopathologic evaluation

Kidney tissues in 12 wk post-ischemic kidneys were stained by hematoxylin and eosin, and the staining was semi-quantitatively graded for tubulointerstitial damage (tubular dilation or atrophy and interstitial expansion with edema, inflammatory infiltrate, or fibrosis) based on a scale of 0–3 as follows: normal cortical tubulointerstitium scored 0; mild tubulointerstitial damage affecting up to 25% of an objective field at ×200 magnification scored 1; moderate tubulointerstitial damage affecting 25%–50% of the field scored 2; and severe tubulointerstitial damage exceeding 50% of the field scored 3. The examiners were blinded to the different groups and the randomly selected 10 cortical fields were scored for each animal and the mean score attributed to the animal [8].

2.6. Immunohistochemistry

Kidney tissue sections (4 μm thick) were subjected to immunohistochemical analysis. Sections were dewaxed in xylene, rehydrated through graded ethanol solutions, rinsed in phosphate-buffered saline for 5 min, and immersed in 3% hydrogen peroxide in methanol for 15 min to block endogenous peroxidase activity. The slides were then rinsed in phosphate-buffered saline for 5 min, blocked with 5% bovine serum albumin at room temperature for 15 min, then incubated with ED-1 antibody (1:50 dilution; Serotec Ltd, Oxford, UK) or transforming growth factor beta 1 (TGF-β1) antibody (1:50 dilution; Santa Cruz Biotechnology, Santa Cruz, CA) at 4°C overnight. Rabbit IgG Isotype was used as the negative control. The slides were then incubated with biotinylated mouse anti-rabbit IgG secondary antibody (Maixin Biotechnology, Fuzhou, China). Finally, the sections were incubated