Original Paper

Effect of Intervention in Mast Cell Function Before Reperfusion on Renal Ischemia-Reperfusion Injury in Rats

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Key Words

Mast cell • Renal ischemia-reperfusion injury • Ketotifen • Cromolyn sodium

Abstract

Background/Aims: Mast cells are sparsely distributed in the kidneys under normal conditions; however, the number of mast cells increases dramatically during renal ischemia/reperfusion injury (RI/RI). When mast cells are stimulated, numerous mediators are released, and under pathological conditions, they produce a wide range of biological effects. The aim of this study was to investigate the effect of intervention in mast cell function before reperfusion on RI/RI. **Methods:** Sprague-Dawley (SD) rats ($n=50$) were randomized into five groups: sham group, ischemia/reperfusion (I/R) group, cromolyn sodium treatment group (CS+I/R group), ketotifen treatment group (K+I/R group), and compound 48/80 treatment group (C+I/R group). I/R injury was induced by bilateral renal artery and vein occlusion for 45 min followed by 24 h of reperfusion. The agents were intravenously administered 5 min before reperfusion through the tail vein. The serum levels of blood urea nitrogen (BUN), serum creatinine (Scr) and histamine and the kidney levels of malondialdehyde (MDA), superoxide dismutase (SOD), tumor necrosis factor α (TNF- α) and interleukin-6 (IL-6) were assessed. The expression of intracellular adhesion molecule-1 (ICAM-1) in renal tissue was also measured. **Results:** I/R injury resulted in severe renal injury, as demonstrated by a large increase in injury scores; serum levels of BUN, Scr and histamine; and kidney levels of MDA, TNF- α , and IL-6; this was accompanied by reduced SOD activity and upregulated ICAM-1 expression. Treatment with cromolyn sodium or ketotifen markedly alleviated I/R-mediated kidney injury, whereas compound 48/80 further aggravated kidney injury. **Conclusion:** Intervention in mast cell activity prior to reperfusion has a strong effect on RI/RI.

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Introduction

Renal ischemia/reperfusion injury (RI/RI) is a common clinical problem that is increasing in prevalence and is associated with higher morbidity and mortality, as well as prolonged length of hospitalization [1]. RI/RI is often associated with an increase in plasma levels of blood urea nitrogen (BUN), serum creatinine (Scr), and numerous inflammatory factors and focal destruction of tubular epithelial cells [2]. Despite extensive studies on RI/RI, the mechanisms behind RI/RI and an effective way to decrease renal injury remain to be elucidated.

Mast cells are ordinarily distributed in normal connective tissue, mainly located adjacent to blood and lymphatic vessels, nerves, and epithelial surfaces, as well as in the skin and in the gastrointestinal and respiratory systems [3]. Under normal circumstances, mast cells are very sparsely distributed in the kidneys, but the number of mast cells increases dramatically during RI/RI [4]. When mast cells are stimulated, numerous mediators, such as histamine, trypsin-like enzyme, chymase, heparin, and a host of cytokines are released from mast cells, and under pathological conditions, they produce a wide array of biological effects [5]. Histamine is an amine substance released by mast cell degranulation, and histamine receptors are significantly involved in allergic reactions, inflammatory responses, and modulation of the body's immune function, as well as as promotion of the secretion of gastric acid and the maintenance of endothelial function [5]. It has been reported that the histamine antagonist ketotifen could improve the survival rate of rats subjected to intestinal ischemia reperfusion injury [6]. Other reports demonstrated that inhibition of inflammatory factors such as TNF- α , IL-6, and ICAM-1, as well as inhibition of lipid peroxidation can all protect kidneys against renal ischemia reperfusion-induced injury [7-9].

In this study, renal injury in rats was induced by bilateral renal artery and vein occlusion followed by reperfusion. Cromolyn sodium (a mast cell membrane stabilizer), ketotifen (a histamine receptor H1 antagonist), and compound 48/80 (a mast cell degranulation agent) were intravenously administered 5 min before reperfusion through the tail vein. The effects of pharmacological intervention in mast cell function before reperfusion on attenuating renal ischemia-reperfusion injury was investigated.

Materials and Methods

Animals and reagents

Sprague-Dawley (SD) rats (180–220 g) were supplied by the Animal Research Center of Shantou University Medical College, Shantou, China. All of the procedures and care administered to the animals were been approved by the Institutional Ethics Committee. Cromolyn sodium, ketotifen, compound 48/80, and other reagents were purchased from Sigma.

Animal experimental design

Fifty SD rats were randomly assigned to five groups (10 rats per group): (1) sham group; (2) I/R group: I/R was induced by bilateral renal artery and vein occlusion for 45 min followed by 24 h of reperfusion; (3) cromolyn sodium treatment group (CS+I/R): 45 min of ischemia followed by 24 h reperfusion with cromolyn sodium pretreatment (25 mg/kg, i.v.) 5 min prior to reperfusion; (4) ketotifen treatment group (K+I/R): 45 min of ischemia followed by 24 h reperfusion with ketotifen pretreatment (1 mg/kg, i.v.) 5 min prior to reperfusion; (5) compound 48/80 treatment group (C+I/R): 45 min of ischemia followed by 24 h reperfusion with compound 48/80 pretreatment (0.75 mg/kg, i.v.) 5 min prior to reperfusion.

Surgical procedures of RI/RI

SD rats were anesthetized with 1% pentobarbital sodium (50 mg/kg) via peritoneal injection. The abdominal cavity was opened, and the renal artery and vein were separated bilaterally. Each artery and vein was occluded for 45 min by two artery clamps, and success was confirmed by observing color change in the

renal tissue. The artery clamps were removed 45min later, and the renal arteries and veins were allowed to reperfuse for 24 h. Blood samples were collected from the inferior vena cava and centrifuged at 3600 rpm for 15 min to harvest the sera. The left kidneys of animals were removed and immediately excised for histopathologic evaluation and injury scores or stored at -20°C for the analyses described below.

Histopathologic evaluation

Four-micron-thick sections of kidneys were cut and stained with hematoxylin and eosin (H&E). Samples were blindly analyzed to determine the extent of kidney injury based on the technique outlined by Erdogan et al. [10]. Briefly, 24 areas corresponding to the kidney proximal tubules were graded for the degree of renal damage based on each of the following parameters: tubular cell necrosis, cytoplasmic vacuole formation, hemorrhage, and tubular dilatation. Specifically, one whole deep coronal section was examined under the microscope and graded according to the extent of damage, based on the percentage of involvement of the kidney. Higher scores represent more severe damage, with the maximum score being 4 [0, histopathological changes $<10\%$; 1, (10%–25%); 2, (25%–50%); 3, (50%–75%); and 4, (75%–100%)]. The mean score for each parameter was determined and subjected to statistical analysis [11, 12].

Assessment of renal function

Blood samples were obtained from the inferior vena cava 24 h after reperfusion. BUN and Scr levels were assayed by the o-phthalaldehyde and picric acid methods for the assessment of renal function [7].

Measurement of superoxide dismutase (SOD) activity and malondialdehyde (MDA) content

The SOD activity and MDA content in renal tissues were measured to assess lipid peroxidation as described previously [7]. Kidney specimens were collected after reperfusion for 24 h and the blood was rinsed off thoroughly. Each kidney was homogenized in ice-cold normal saline and then centrifuged at 3600 rpm for 10 min to harvest the supernatant. The content of total protein in the supernatant was measured by Coomassie brilliant blue method as described by the manufacturer's protocol. The SOD activity and MDA content were measured by the xanthine oxidase and thiobarbituric acid methods. The level of lipid peroxides was expressed as U of SOD/mg protein and nmol of MDA/mg protein.

Measurement of tumor necrosis factor α (TNF- α) content

The quantity of TNF- α in renal tissues was measured to assess inflammatory injury as described previously [13]. The TNF- α content was measured using the double-antibody sandwich ELISA method. The absorbance was measured at 495 nm. The content of inflammatory injury was expressed as ng of TNF- α /g protein.

Measurement of interleukin-6 (IL-6) content

The quantity of IL-6 in renal tissues was measured to assess inflammatory injury as described previously [14, 15]. The sampling process was the same as above and the IL-6 content was measured using the double-antibody sandwich ELISA method. The absorbance was measured at 450 nm. The content of inflammatory injury was expressed as pg of IL-6/mg protein.

Determination of histamine in serum

At the pre-determined time, a 0.3-mL blood sample was obtained from the inferior vena cava 24 h after reperfusion. Histamine content in plasma was determined by the use of a histamine assay kit as described previously [4].

Western blot analysis

Western blot analysis was conducted as described previously [16]. Briefly, renal tissues were homogenized in protein lysate buffer. The homogenates were resolved on polyacrylamide SDS gels, and electrophoretically transferred to polyvinylidene difluoride membranes. The membranes were blocked with 3% BSA, incubated with primary antibodies against active intracellular adhesion molecule-1 (ICAM-1) and then with alkaline phosphatase-conjugated secondary antibody. The membranes were subsequently

Fig. 1. Histopathologic evaluation of kidney injury. Light microscope images ($\times 400$) a, b, c, d, and e correspond to sham group, I/R group, CS+I/R group, K+I/R group, and C+I/R group, respectively. Quantitative injury scores, expressed as the mean $\pm$ SD, are shown in Fig. 1f. A significant increase relative to the sham group is denoted by “*” ($p < 0.01$), a significant decrease relative to the I/R group is denoted by “#” ($p < 0.05$), and a significant increase relative to the I/R group is denoted by “##” ($p < 0.01$).

developed by 5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium. Blots were stained with anti- β -actin antibodies, and protein levels were normalized with respect to β -actinband density.

Statistical analysis

All data were expressed as the mean value $\pm$ standard deviation (SD). Statistical analysis between groups was carried out using ANOVA with post-hoc testing and Pearson correlation analysis. A value of less than 0.01 ($P < 0.01$) was used for statistical significance.

Results

Histopathologic evaluation

Light microscopy images of kidney sections are shown in Fig. 1. Tubular cell necrosis, cytoplasmic vacuoles, hemorrhage, and tubular dilatation were observed in histological specimens from the I/R group (Fig. 1b) but were absent in the sham group (Fig. 1a). Histological alterations were markedly reduced in specimens from the cromolyn sodium- and ketotifen-treated groups (Fig. 1c and 1d) compared to the I/R group. Histological alterations were increased in specimens from the compound 48/80-treated group (Fig. 1e) compared with the I/R group. The corresponding quantitative analysis is shown in Fig. 1f.

Serum level of BUN and Scr

As shown in Fig. 2, the serum levels of BUN and Scr in RI/RI rats were significantly higher than those in sham rats. The level of BUN was 6.45 ± 0.48 mmol/L in sham rats, whereas the level of BUN reached 24.13 ± 0.98 mmol/L in the I/R rats. Administration of cromolyn sodium and ketotifen significantly reduced the levels of BUN (16.78 ± 1.08 mmol/L for the CS+I/R group and 16.03 ± 0.85 mmol/L for the K+I/R group), whereas administration of compound

Fig. 2. Serum level of BUN and Scr. The results are expressed as the mean $\pm$ SD. A significant increase relative to the sham group is denoted by "*" ($p < 0.01$), a significant decrease relative to the I/R group is denoted by "#" ($p < 0.05$), and a significant increase relative to the I/R group is denoted by "##" ($p < 0.01$).

48/80 significantly increased the level of BUN (31.21 ± 1.21 mmol/L) compared with that of I/R rats. The level of Scr was 45.13 ± 1.85 μ mol/L in sham rats, whereas the level of Scr reached 151.27 ± 2.11 μ mol/L in I/R rats. Administration of cromolyn sodium and ketotifen significantly reduced the levels of Scr (130.16 ± 1.28 μ mol/L for the CS+I/R group and 125.06 ± 1.65 μ mol/L for the K+I/R group), whereas administration of compound 48/80 increased the levels of Scr (168.88 ± 3.09 μ mol/L) compared with I/R rats.

The activity of SOD and the level of MDA in renal tissues

The activities of SOD and the levels of MDA in renal tissues are shown in Fig. 3. The activity of SOD in renal tissues induced by I/R (18.78 ± 1.19 U/mg protein) was significantly less than that in the sham group (53.25 ± 2.87 U/mg protein). Administration of cromolyn sodium and ketotifen significantly increased the activity of SOD (31.19 ± 2.13 U/mg protein for the CS+I/R group and 30.08 ± 1.24 U/mg protein for the K+I/R group) in renal tissues compared with that in the I/R group. Administration of compound 48/80 decreased the activity of SOD

Fig. 3. The activity of SOD and the level of MDA in renal tissues. The results are expressed as the mean $\pm$ SD. (a) A significant decrease relative to the sham group is denoted by "*" ($p < 0.01$), a significant increase relative to the I/R group is denoted by "#" ($p < 0.05$), and a significant decrease relative to the I/R group is denoted by "##" ($p < 0.01$). (b) A significant increase relative to the sham group is denoted by "*" ($p < 0.01$), a significant decrease relative to the I/R group is denoted by "#" ($p < 0.05$), and a significant increase relative to the I/R group is denoted by "##" ($p < 0.01$).

(14.17 ± 2.05 U/mg protein) compared with that in the I/R group. The level of MDA in renal tissues in the I/R group (10.08 ± 0.27 nmol/mg protein) was significantly higher than that in the sham group (3.05 ± 0.16 nmol/mg protein). Administration of cromolyn sodium and ketotifen significantly reduced the level of MDA (6.05 ± 0.43 nmol/mg protein for the CS+I/R group and 6.27 ± 0.38 nmol/mg protein for the K+I/R group) in renal tissues, compared with the level of MDA in I/R group. Administration of compound 48/80 increases the level of MDA (13.15 ± 0.32 nmol/mg protein), compared with that in the I/R group.

The level of TNF- α and IL-6 in renal tissues

The levels of TNF- α in renal tissues are shown in Fig. 4a. The level of TNF- α induced by I/R (1.132 ± 0.0125 ng/g protein) was significantly higher than that in the sham group (0.3617 ± 0.0118 ng/g protein). Administration of cromolyn sodium and ketotifen significantly reduced the level of TNF- α (0.6117 ± 0.0132 ng/g protein for the CS+I/R group and 0.6198 ± 0.0132 ng/g protein for the K+I/R group) in renal tissues, compared with the level of TNF- α in the I/R group. Administration of compound 48/80 increased the level of TNF- α (1.347 ± 0.0128 ng/g protein), compared with that in the I/R group. The levels of IL-6 in renal tissues are demonstrated in Fig. 4b. The level of IL-6 induced by I/R (80.13 ± 2.31 pg/mg protein) was significantly higher than that in the sham group (9.18 ± 1.05 pg/mg protein). Administration of cromolyn sodium and ketotifen significantly reduced the level of IL-6 (56.18 ± 1.29 pg/mg protein for the CS+I/R group and 57.72 ± 1.83 pg/mg protein for the K+I/R group) in renal tissues compared with the level of IL-6 in the I/R group. Administration of compound 48/80 increased the level of IL-6 (91.23 ± 2.19 pg/mg protein) compared with that in the I/R group.

Changes in serum histamine levels

The levels of serum histamine for different groups are shown in Fig. 5. The level of histamine induced by I/R (167.24 ± 2.16 nM) was significantly higher than that in the sham group (46.13 ± 1.04 nM). Administration of cromolyn sodium and ketotifen reduced the level of histamine (146.15 ± 2.27 nM for the CS+I/R group and 145.83 ± 1.98 nM for the K+I/R group) in serum compared with the level of histamine in the I/R group. Administration of compound 48/80 significantly increased the level of histamine (196.37 ± 2.45 nM) compared with that in the I/R group.

Fig. 4. The level of TNF- α and IL-6 in renal tissues. The results are expressed as the mean $\pm$ SD. A significant increase relative to the sham group is denoted by “*” ($p < 0.01$), a significant decrease relative to the I/R group is denoted by “#” ($p < 0.05$), and a significant increase relative to the I/R group is denoted by “##” ($p < 0.01$).

Fig. 5. The concentration of histamine in plasma. The results are expressed as the mean $\pm$ SD. A significant increase relative to the sham group is denoted by “*” ($p < 0.01$), a significant decrease relative to the I/R group is denoted by “#” ($p < 0.05$), and a significant increase relative to the I/R group is denoted by “##” ($p < 0.01$).

Fig. 6. The expression of ICAM-1 in renal tissues. The results are expressed as the mean $\pm$ SD. A significant increase relative to the sham group is denoted by “*” ($p < 0.01$), a significant decrease relative to the I/R group is denoted by “#” ($p < 0.05$), and a significant increase relative to the I/R group is denoted by “##” ($p < 0.01$).

The expression of ICAM-1 in renal tissues

The expression of ICAM-1 in renal tissues is shown in Fig. 6. It can be seen that the expression of ICAM-1 induced by I/R was significantly higher than in the sham group. Administration of cromolyn sodium and ketotifen significantly downregulated the expression of ICAM-1 in renal tissues compared with the expression of ICAM-1 in the I/R group. Administration of compound 48/80 significantly upregulated the expression of ICAM-1 compared with that in the I/R group.

Correlation analysis

Correlation analysis amongst histamine, BUN, Scr, SOD, MDA, TNF- α , IL-6 content and ICAM-1 expression was conducted. The results showed that the serum histamine level had a positive correlation with serum BUN ($r = 0.817$, $p < 0.01$) and Scr levels ($r = 0.831$, $p < 0.01$) and that serum BUN and Scr levels had a negative correlation with SOD activity ($r = -0.654$, $p < 0.01$) but a positive correlation with MDA levels ($r = 0.732$, $p < 0.01$), TNF- α levels ($r = 0.802$, $p < 0.01$), IL-6 levels ($r = 0.884$, $p < 0.01$) and ICAM-1 expression ($r = 0.706$, $p < 0.01$).

Discussion

Altering mast cell activity and function has proven to be a promising technique to reduce intestine, lung, heart, and brain ischemia reperfusion injury [6, 17-20]. It has been reported that cromolyn sodium (a mast cell stabilizer) can interfere with mast cell function to reduce lung damage during the early secondary lung injury phase of acute pancreatitis and that mast cells could alleviate organ damage by increasing the expression of neutrophils, monocytes, and ICAM-1 [21]. Previous studies demonstrated that cromolyn sodium, working as a mast cell stabilizer, and ketotifen, working as a histamine receptor H_1 antagonist, decreased intestinal ischemia reperfusion injury, while compound 48/80, which works as a mast cell degranulation agent, aggravated intestinal ischemia reperfusion injury [22]. In the present study, renal ischemia reperfusion led to renal damage and was accompanied by elevated

serum BUN and Scr; administration of cromolyn sodium and ketotifen attenuated I/R injury, whereas compound 48/80 exacerbated I/R injury.

Histamine, one of the main active substances produced by mast cells, can increase vascular permeability, induce inflammatory cell infiltration, stimulate epithelial cells, and release cytokines, all of which cause or exacerbate tissue damage that can further induce mast cell activation. Histamine antagonists are widely used in protection from ischemia reperfusion injury, and some studies show that ketones can decrease intestinal ischemia reperfusion injury, while compound 48/80 can increase damage [5, 6]. Our results showed that I/R increased serum histamine, while cromolyn sodium and ketotifen reduced serum histamine and compound 48/80 exhibited the opposite effect. At the same time, correlation analysis results demonstrated that serum histamine concentrations had a positive correlation with serum BUN and Scr levels. The above results suggest that endogenous mast cells are activated after renal ischemia reperfusion and subsequently release a large number of active substances such as histamine that damage kidney tissues.

Renal I/R has been demonstrated to involve the production of reactive oxygen species (ROS) and dependent signaling cascades [23-26]. It has been reported that mast cells can increase oxidative damage and promote the production of ROS, which reduce the activity of SOD and increase MDA levels [27]. The present study demonstrated that cromolyn sodium and ketotifen both improved SOD activity and decreased MDA content with reduced renal oxidative injury, while compound 48/80 displayed the opposite effect, leading to increased I/R damage. Correlation analysis results showed that serum BUN and Scr had a negative correlation with SOD activity but a positive correlation with MDA content. The above results suggest that mast cells may participate in I/R renal injury through mediating oxidative stress.

I/R injury involves the inflammatory response of both the innate and adaptive immune systems through exaggerated inflammatory cell infiltration and tubular epithelial cell activation [28-30]. When renal I/R injury occurs, inflammatory cells initially infiltrate into the damaged renal tissue and promote marked pro-inflammatory mediator secretion; the latter promotes the infiltration of inflammatory cells into the damaged tissues and further promotes inflammatory tissue responses [31, 32], which results in a vicious positive feed back loop. Furthermore, infiltrated inflammatory cells reduce renal blood flow, which leads to microcirculatory dysfunction [33, 34]. Our results showed that I/R injury induced the production of TNF- α , IL-6, and ICAM-1. Tissue histology showed significant pathology, with renal tissue bleeding, edema, and inflammatory cell infiltration. Serum BUN, Scr were also significantly increased. Administration of cromolyn sodium and ketotifen reduced the level of TNF- α , IL-6 and downregulated the expression of ICAM-1, while compound 48/80 had the opposite effect. These results imply that inhibition of mast cell degranulation and antagonistic histamine effects both can reduce I/R injury whereas promotion of mast cell degranulation can aggravate I/R injury. Correlation analysis results showed that serum BUN and Scr had a positive correlation with TNF- α , IL-6 and ICAM-1. These results showed that the activation of mast cells is involved in the production of proinflammatory factors, which also contributed to renal I/R injury.

Conclusion

In summary, our results indicate that pharmacological modulation of mast cell activity significantly altered renal ischemia-reperfusion injury. The inhibition of mast cell function before reperfusion and the reduction of histamine release alleviated oxidative damage and the inflammatory response, protecting the kidney from I/R injury.

Disclosure Statement

Nothing to declare.

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