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ORIGINAL ARTICLE

Protective effects of probucol in rats with postoperative acute renal failure*

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Abstract

Objective We investigated the protective effect of probucol in rats with acute renal failure caused by various ischemia-reperfusion injuries (IRIs) after surgery.

Methods Forty male Sprague-Dawley rats were randomly divided into a sham operation group (S group), ischemia reperfusion group (IR group), probucol low-dose treatment group (probucol + IR group 1, P+ IR 1 group; probucol 250 mg/kg intragastric administration daily), and probucol high-dose treatment group (P + IR 2 group; probucol 500 mg/kg intragastric administration daily). Rats in the S and IR groups were intragastrically administered with warm water every day. After 1 week, the kidney IRI rat models were prepared, after which the rats were fed for another week, and blood, urine, and the kidney tissue specimens were retained. A series of biochemical indices, superoxide dismutase (SOD), and malondialdehyde in the serum and kidney tissues were detected, and pathological changes in renal tissue were observed.

Results Twenty-four-hour urinary protein excretion, urinary NAGase, CysC, blood urea nitrogen (BUN), and creatinine were significantly lower in the P + IR 1 and P + IR 2 groups than in the IR group ($P < 0.05$). Superoxide dismutase in the serum and renal tissue increased significantly, malondialdehyde decreased significantly ($P < 0.05$), renal pathological injury was alleviated, and the kidney index improved significantly ($P < 0.05$).

Conclusion Probucol can relieve various types of acute renal failure in postoperative rats.

Key words: probucol; ischemia-reperfusion; acute renal failure; oxidative stress

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Clinically, patients with various organ tumors often experience acute renal failure after surgery, which is among the most important causes of death after tumor surgery. The choice of effective drugs for preventing and treating acute renal failure and reducing the mortality rate of patients after tumor surgery has been widely examined. Renal ischemia-reperfusion injury (IRI) is one of the common causes of acute renal failure [1]. The kidney is an organ exhibiting high blood perfusion [2] and is very sensitive to ischemia. Excess oxygen free radicals during reperfusion further damage the renal tissue [3]. In addition to its lipid-lowering effect, probucol has anti-inflammatory, anti-oxidative, and other effects. Our previous studies confirmed that probucol has a protective effect in rats with doxorubicin nephropathy [4–5]. This study examined whether the drug also has protective effects in rats with IRI-induced acute renal failure.

Materials and methods

Experimental animals

Forty healthy male Sprague-Dawley (SD) rats weighing 180–220 g were provided by the Animal Experimental Center of Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China (Experimental Animal Production License No.: SYXK (E) 2014-0046). After feeding for one week in separate cages at room temperature, urinary protein test results were all negative.

Drugs and reagents

The probucol tablets (Changtai) were produced by Chengde Jing Fu Kang Pharmaceuticals Co., Ltd. (China). The strength was 0.25 g/tablet and the lot number was 070801. Serum superoxide dismutase (SOD, lot No. 080323) and malondialdehyde (MDA, lot No. 080214) detection kits were purchased from Wuhan Kerui Biotech

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Co., Ltd. (Wuhan, China).

Grouping and model preparation

Forty rats were randomly divided into the sham operation group [S group; body weight (205 ± 8.1) g], ischemia-reperfusion group [IR group; body weight (207 ± 9.3) g], probucol low-dose treatment group [probucol + IR 1 group, P + IR 1 group; body weight (208 ± 7.9) g], and probucol high-dose treatment group [P + IR 2 group; body weight (207 ± 6.5) g]. Rats in the S and IR groups were intragastrically administered warm water daily. The P + IR 1 group was intragastrically administered probucol 250 mg/(kg•d), and the P + IR 2 group was intragastrically administered probucol 500 mg/(kg•d). IRI rat models were prepared after 1 week. The four groups of rats were fasted for 12 h before surgery. Anesthesia was performed by intraperitoneal injection of 3.5% chloral hydrate (l mL/100 g). An incision was made on the lower abdomen along the midline through the xiphoid process, the right kidney was exposed, and the right kidney vein was punctured to collect blood, after which the right kidney was excised. For the S group, only excision of the right kidney and separation of the left renal artery and vein were performed, while the left renal artery was not clipped. The left kidney was exposed, and the left renal artery and vein were separated. The left renal artery was clipped with a non-invasive arterial clip, and the color of the kidney changed from bright red to dark red. After 30 min of ischemia, the arterial clip was released and the color of the kidney changed from dark red to bright red, indicating that reperfusion was successful and the model was successfully established [6]. Normal saline replacement was administered intraperitoneally, the wound was closed layer-by-layer and covered with wet gauze, and the abdominal cavity was closed.

Specimen collection

After the models were established, the S and IR groups were intragastrically administered warm water every day, and the P + IR 1 and P + IR 2 groups were intragastrically administered probucol every day for 1 week. Rats were individually fed in metabolic cages before and on days 1, 4, and 7 after modeling. The rats were fasted without water deprivation, and 24-h urine was collected. Blood was drawn from the caudal vein. On day 7, the blood and kidney tissue samples were taken from all rats. An abdominal incision was made to remove the kidneys under sterile conditions. A portion of renal tissue was washed with normal saline at 4 °C, weighed, and ground, and the homogenate was centrifuged at 4100× g and 4 °C to obtain the supernatant for determination of SOD and MDA activity. The other part of the kidney tissue was examined under a light microscope to evaluate histomorphological changes.

Observation indicators

The kidneys were weighed, and the kidney index was calculated: (kidney weight / body weight) × 10^{-2}. Urine test: 24-h urinary protein quantification and urinary N-acetyl-beta-D-glucosaminide (NAG) enzyme were tested by the Clinical Laboratory of Wuhan General Hospital of PLA, China.

Blood test: Serum creatinine (SCr), blood urea nitrogen (BUN), and cystatin C (CysC) were measured 1 week after the models were established. Measurements were performed with an automatic biochemistry analyzer by the Clinical Laboratory of Wuhan General Hospital of PLA (China) and the average of two measurements was taken.

Serum and kidney tissue SOD and MDA detection: SOD activity and MDA content in the serum and kidney tissue were measured 1 week after the models were established. The specific procedures and calculation formulas were conducted according to the kit instructions, and the average of two measurements was taken.

Kidney morphology examination: Light microscopy: the kidney tissue was obtained, fixed with 10% formaldehyde solution, dehydrated conventionally, embedded with paraffin, and cut into 2–4-μm slices for hematoxylin and eosin (HE), Masson, and silver staining, and was observed under a regular light microscope and photographed with a professional camera.

Statistical methods

The experimental results were expressed as the mean ± the standard deviation ($\bar{\chi} \pm s$). The measurement data of multiple groups were compared by analysis of variance and categorical data were compared using χ^2 test with SPSS 11.3 software (SPSS, Inc., Chicago, IL, USA). The difference was considered statistically significant at $P < 0.05$.

Results

General conditions of rats

Compared to the S group, rats in the IR, P + IR 1, and P + IR 2 groups showed gradually slowing reactions, reduced movement, crouching and arched back, poor hair color, reduced eating and drinking, edema in the lips and limbs, and an enlarged abdomen. In the P + IR 1 and P + IR 2 groups, 1 week after treatment with probucol, food and water intake improved, body weight began to increase, reactions improved, and edema decreased, but the values were still worse than those for the S group. There was no significant improvement in the IR group.

Changes in 24-h urinary protein and urinary NAG enzyme levels before and after treatment in all groups of rats

The 24-h urinary protein and urinary NAG enzyme levels in the IR, P + IR 1, and P + IR 2 groups were significantly higher than those in the S group after modeling ($P < 0.05$). Compared to IR rats, the 24-h urinary protein and urinary NAG enzyme levels in the P + IR 1 and P + IR 2 groups were significantly reduced. The 24-h urinary protein and urine NAG enzyme levels in the P + IR 2 group were lower than those in the P + IR 1 group, but the difference was not significant ($P > 0.05$; Tables 1–2).

Changes of blood biochemistry and kidney index in each group

The levels of CysC, SCr, and BUN in the P + IR 1 and P + IR 2 groups were significantly lower than those in the IR group ($P < 0.05$). The levels of CysC, SCr, and BUN in the P + IR 2 group were lower than those in the P + IR 1 group, but the difference was not significant ($P > 0.05$). The renal index level was significantly improved in the P + IR 1 and P + IR 2 groups compared to that in the IR group (Table 3).

Contents of SOD and MDA in the serum and kidney tissue of each group

The content of MDA in serum and renal tissue of the IR group was significantly increased, while the activity of SOD was significantly decreased. Compared to the IR

Table 1 Comparison of 24-h urinary protein levels in each group ($\bar{\chi} \pm s$, mg)

Group (Number of animals)	Before modeling	1 day after modeling	4 days after modeling	7 days after modeling
S (10)	6.32 ± 1.27	6.45 ± 1.08	6.62 ± 1.35	6.62 ± 1.35
IR (10)	6.71 ± 1.85	182.12 ± 15.24^{ab}	279.12 ± 19.61^{ab}	373.12 ± 22.61^{ab}
P + IR 1 (10)	6.64 ± 1.57	132.36 ± 19.27^{abc}	210.17 ± 13.26^{abc}	302.38 ± 21.74^{abc}
P + IR 2 (10)	6.62 ± 1.43	125.65 ± 20.36^{abcd}	203.45 ± 14.05^{abcd}	294.45 ± 21.05^{abcd}

Note: Compared to the value before modeling, $^{a}P < 0.05$; compared to the S group, $^{b}P < 0.05$; compared to the IR group, $^{c}P < 0.05$; compared to the P + IR 1 group, $^{d}P > 0.05$

Table 2 Comparison of urine NAG enzyme levels in each group ($\bar{\chi} \pm s$, U/L)

Group (Number of animals)	Before modeling	1 day after modeling	4 days after modeling	7 days after modeling
S (10)	20.4 ± 2.3	20.7 ± 1.9	21 ± 2.4	22 ± 2.2
IR (10)	20.5 ± 2.1	49.1 ± 3.9^{ab}	74.4 ± 4.3^{ab}	90.4 ± 5.7^{ab}
P + IR 1 (10)	20.3 ± 2.4	40.5 ± 3.4^{abc}	53.7 ± 4.1^{abc}	76.1 ± 5.2^{abc}
P + IR 2 (10)	20.3 ± 2.2	38.6 ± 3.1^{abcd}	49.1 ± 3.8^{abcd}	72.5 ± 4.9^{abcd}

Note: Compared to the value before modeling, $^{a}P < 0.05$; compared to the S group, $^{b}P < 0.05$; compared to the IR group, $^{c}P < 0.05$; compared to the P + IR 1 group, $^{d}P > 0.05$

Table 3 Effects on blood biochemistry and kidney index in each group ($\bar{\chi} \pm s$)

Group (Number of animals)	CysC (mg/L)	BUN (mmol/L)	SCr (mmol/L)	Kidney index
S (10)	0.87 ± 0.26	7.23 ± 1.13	57.38 ± 11.72	0.82 ± 0.35
IR (10)	4.68 ± 1.75^{a}	10.58 ± 2.35^{a}	146.13 ± 10.45^{a}	1.26 ± 0.35^{a}
P + IR 1 (10)	3.01 ± 0.84^{ab}	9.5 ± 1.22^{ab}	102.35 ± 9.82^{ab}	1.054 ± 0.43
P + IR 2 (10)	2.91 ± 0.73^{abc}	9.4 ± 1.02^{abc}	98.16 ± 9.74^{abc}	1.04 ± 0.41^{abc}

Note: Compared to the S group, $^{a}P < 0.05$; compared to the IR group, $^{b}P < 0.05$; compared to the P + IR 1 group, $^{c}P > 0.05$

Table 4 SOD and MDA contents in the serum and kidney tissue of each group ($\bar{\chi} \pm s$)

Group (Number of animals)	Serum SOD (U/mL)	Kidney tissue SOD (U/mL)	Serum MDA (nmol/mL)	Kidney tissue MDA (nmol/mL)
S (10)	60.53 ± 11.63	97.85 ± 16.49	2.25 ± 0.73	2.87 ± 1.25
IR (10)	32.76 ± 7.72^{a}	49.12 ± 11.46^{a}	4.61 ± 1.26^{a}	4.91 ± 1.67^{a}
P + IR 1 (10)	52.13 ± 10.79^{ab}	84.28 ± 16.47^{ab}	3.96 ± 1.82^{ab}	4.11 ± 1.52^{ab}
P + IR 2 (10)	49.02 ± 10.24^{abc}	82.31 ± 15.98^{abc}	3.87 ± 1.73^{abc}	3.97 ± 1.35^{abc}

Note: Compared to the S group, $^{a}P < 0.05$; compared to the IR group, $^{b}P < 0.05$; compared to the P + IR 1 group, $^{c}P > 0.05$

group, the content of MDA in the serum and kidney tissue of the P + IR 1 and P + IR 2 groups decreased significantly, and SOD increased significantly ($P < 0.05$). The content of MDA in the P + IR 2 group was lower than that in the P + IR 1 group and the SOD was increased, but the difference was not significant ($P > 0.05$; Table 4).

Changes of kidney pathomorphology in each group

Under light microscopy, the glomeruli, renal tubules, and interstitium were normal in the S group. The IR group was mainly characterized by renal tubular injury. HE staining showed slight hyperplasia of the glomerular mesangial matrix, widened renal interstitium infiltrated by a considerable volume of inflammatory cells, some atrophied renal tubules, and shedding tubular epithelial cells; a considerable volume of red blood cells and white blood cells in the renal interstitium, as well as blood stasis in the small interstitial blood vessels were observed. Masson + silver staining showed a focal distribution of interstitial fibrosis. The pathological changes of rats in the P + IR 1 and P + IR 2 groups were significantly lower

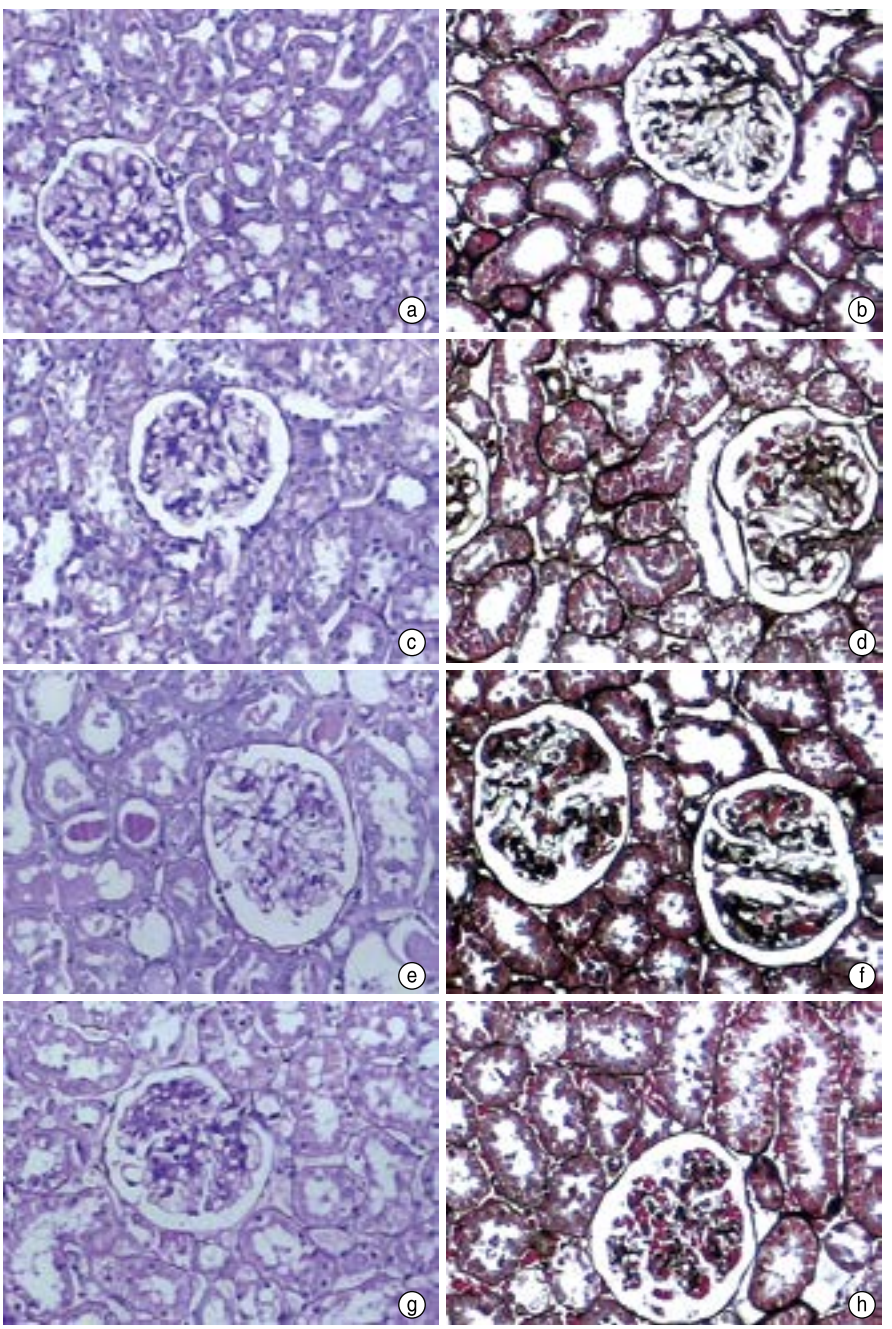

Fig. 1 Pathomorphological changes in the kidneys of rats in each group (×400). (a) HE staining of the S group; (b) Masson + silver staining of the S group; (c) HE staining of the IR group; (d) Masson + silver staining of the IR group; (e) HE staining of the P + IR 1 group; (f) Masson + silver staining of the P + IR 1 group; (g) HE staining of the P + IR 2 group; (h) Masson + silver staining of the P + IR 2 group

than those in the IR group. The glomerular structure was relatively normal, and renal interstitial lesions were mild (Fig. 1).

Discussion

As patients with advanced malignant tumors, particularly elderly patients, have reduced immune function and degenerative changes in the function of various organs, poor tolerance to surgery and acute renal failure after surgery are common. This is mainly because blood loss after surgery leads to effective circulating blood volume, microcirculation, renal ischemia, and hypoxia injury; vital organs of the body, including the kidneys, have reduced compensatory ability; and surgical wounds lead to increased oxidative stress and increased inflammatory reactivity. Studies have shown that for acute renal failure in elderly patients with malignant tumors, the risk of death is significantly increased. Therefore, effective measures must be taken to prevent and treat acute renal failure in patients after tumor surgery.

The kidney is a high-blood-perfusion organ, whose blood flow accounts for 20%–25% of the blood flow in the body and is very sensitive to ischemia. Kidney IRI is one of the common causes of acute renal failure. The pathogenesis of renal IRI is complex and is currently thought to be mainly related to the following factors: renal cell apoptosis, oxidative stress response, inflammatory response, endothelial dysfunction, and impaired cellular energy metabolism [7–8]. Increased oxidative stress in the kidney is particularly critical. After IRI in the kidney, more oxygen radicals are produced through the xanthine oxidase pathway. Activated oxygen radicals act on the unsaturated fatty acids in the cell membrane of the kidney, resulting in lipid peroxidation and a large amount of MDA. MDA is a commonly used indicator that reflects the body's lipid peroxide content and degree of attack by superoxide radicals [9]. SOD is a major antioxidant enzyme in the body and kidney tissues. It removes superoxide anions from the body, reduces oxidative stress damage in cells, and repairs damaged cells [10–11].

Probucol was used clinically as a lipid-lowering drug in the 1970s. In recent years, studies have shown that probucol also has good anti-oxidative stress, anti-inflammatory, and endothelial function improvement effects. In this study, 24-h urinary protein, urinary NAGase, CysC, SCr, BUN, and other indicators were significantly elevated in rats after IRI, renal pathological lesions were evident, renal index deteriorated, and acute renal failure occurred. After treatment with low-dose and high-dose, the levels of MDA in the serum and renal tissue of the P + IR 1 and P + IR 2 groups were significantly decreased, and SOD activity was significantly increased ($P < 0.05$). The drug improved oxidative stress in the kidneys and restored the balance between oxidation and anti-oxidation. In terms of renal function, 24-h urinary protein quantification, urine NAGase, CysC, SCr, BUN, and other indicators were significantly decreased ($P < 0.05$), indicating that renal failure was improved. In addition, renal pathological injury and the kidney index also significantly improved. These results demonstrate that probucol reduces acute renal failure. Our previous studies confirmed that heme oxygenase-1 (HO-1) has a protective effect on the kidneys in rats with chronic renal insufficiency [12], while probucol induces HO-1 expression and increases the activity of HO-1 [13], which may be another important mechanism in renal protection.

In summary, when acute renal failure occurs after surgery for malignant tumors, probucol has good alleviation and treatment effects. The mechanism may involve lipid regulation, anti-oxidative stress, anti-inflammation, endothelial function improvement, etc. Probucol shows potential for clinical application.

Conflicts of interest

The authors indicated no potential conflicts of interest.

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