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STOBADINE PROTECTS RAT KIDNEY AGAINST ISCHAEMIA/REPERFUSION INJURY

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SUMMARY

1. Ischaemia–reperfusion (I/R) injury, one of the main causes of acute renal failure, still needs satisfactory treatment for routine clinical application. Stobadine, a novel synthetic pyridoindole anti-oxidant, has the ability to reduce tissue injury induced by mechanisms involving reactive oxygen species during I/R. The aim of the present study was to determine the effects of stobadine on renal I/R injury.

2. Forty male Wistar rats were randomly divided into four groups as follows: sham, I/R, stobadine treated and I/R + stobadine treated. Stobadine (2 mg/kg, i.v.) was given intravenously to two groups of rats. The stobadine-treated group was treated with stobadine following sham operation before the abdominal wall was closed, whereas the I/R + stobadine group received stobadine at the beginning of reperfusion. Renal I/R was achieved by occluding the renal arteries bilaterally for 40 min, followed by 6 h reperfusion. Immediately thereafter, blood was drawn and tissue samples were harvested to assess: (i) serum levels of blood urea nitrogen and creatinine; (ii) serum and/or tissue levels of malondialdehyde (MDA), glutathione (GSH), glucose 6-phosphate dehydrogenase (G-6PD), 6-phosphogluconate dehydrogenase (6-PGD), glutathione reductase (GR) and glutathione peroxidase (GPx); (iii) renal morphology; and (iv) immunohistochemical staining for P-selectin.

3. Stobadine was able to significantly attenuate the renal dysfunction as a result of renal I/R injury. Iscahemia–reperfusion resulted in a significant increase in serum and kidney MDA levels and a decrease in serum and kidney GSH. Stobadine treatment at the beginning of reperfusion attenuated both the increased MDA levels and decreased GSH secondary to I/R injury. In addition, the decreased G-6PD activity observed after I/R was significantly attenuated by stobadine treatment. Stobadine did not alter 6-PGD activity after I/R. Neither GR nor GPx activity was significantly changed in the I/R alone or the I/R + stobadine groups compared with the sham group. In addition, stobadine decreased the morphological deterioration and high P-selectin immunoreactivity secondary to renal I/R injury.

4. A pyridoindole anti-oxidant, stobadine exerts a renal protective effect in renal I/R injury, which is probably due to its radical-scavenging and anti-oxidant activities.

Key words: glutathione, ischaemia, kidney, malondialdehyde, reperfusion, stobadine.

INTRODUCTION

Ischaemia–reperfusion (I/R) injury, one of the leading causes of acute renal failure, is unavoidable in some surgical procedures, such as kidney transplantation, abdominal aortic aneurysm repair and renal artery surgery, as well as in certain clinical situations, including various hypotensive states and shock.[1] Nevertheless, there is no satisfactory routine clinical treatment to prevent renal I/R injury.[2] Reperfusion is responsible for most of the injury seen in I/R. Reactive oxygen species (ROS) that are generated during reperfusion are known to be important factors involved in the pathophysiology of renal I/R injury.[3] Two common markers have been used as indicators of oxidative damage in renal I/R injury: (i) malondialdehyde (MDA), which is an end-product of lipid peroxidation; and (ii) glutathione (GSH), which is a key indicator of endogenous anti-oxidant levels.[4,5] Other important molecules include glutathione reductase (GR), which is an enzyme responsible for maintaining cellular GSH pools, and glutathione peroxidase (GPx), which degrades low concentrations of H_2O_2. Glucose-6-phosphate dehydrogenase (G-6PD) and 6-phosphogluconate dehydrogenase (6-PGD), fundamental enzymes of the pentose phosphate pathway and the principal sources of NADPH, serve as anti-oxidant enzymes that modulate the redox milieu.[6] There is increasing evidence that suggests that anti-oxidant compounds are important in preventing I/R injury related damage in the kidney.[7,8]

Stobadine, (–)-*cis*-2,8-dimethyl-2,3,4,4a,5,9b-hexahydro-1H-pyrido-[4,3-b] indole, is a novel synthetic pyridoindole compound. Stobadine is able to diminish lipid peroxidation and protein impairment under conditions of oxidative stress by various mechanisms, including the scavenging of hydroxyl, peroxyl and alkoxyl radicals, quenching singlet oxygen, repairing oxidized amino acids and preventing the oxidation of SH groups.[9] In streptozotocin-induced diabetic rats, stobadine attenuated deterioration of renal function,[10]

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reduced oxidative damage[10] and histochemical changes[11] of kidney tissue, as well as attenuating alterations in activities of kidney GPx and catalase.[12] It has been shown that stobadine also has protective effects in the myocardium, aorta[13] and neural tissue against I/R and hypoxia/re-oxygenation injury or in the presence of chemical systems that generate free oxygen radicals.[9] To the best of our knowledge, only oral administration of the acyl derivative of stobadine, namely heptanoylstobadine, has been assessed as a treatment against renal I/R injury and these studies have used different methodological approaches.[14,15]

The aim of the present study was to evaluate the effect of the intravenous administration of stobadine on renal function and morphology, serum and kidney levels of MDA, GSH and GSH-dependent enzymes and the pentose phosphate pathway enzymes after 40 min ischaemia and 6 h reperfusion in rats *in vivo*.

METHODS

The study was approved by the Animal Ethics Committee of Gazi University and performed in accordance with the guidelines of the Research Committee of Gazi University.

Animals

Forty male Wistar rats, weighing 220–250 g, were acquired from the university's vivarium sources and housed in eight cages at a constant temperature (24°C), humidity (70%) and light–dark cycle in a controlled environment. Rats were fed standard rat chow and tap water *ad libitum*.

Experimental protocols

Rats were randomly divided into four groups as follows: sham ($n = 10$), I/R without stobadine ($n = 10$), stobadine treated without I/R ($n = 10$) and I/R + stobadine treated ($n = 10$). The mean weight of rats was approximately 230 g in all four groups.

Surgical procedure

Following a 12 h fasting period, rats underwent surgery, using ketamine HCl anaesthesia (50 mg/kg) and sterile technique. The abdomen was entered through a midline incision and both renal arteries visualized. In the sham group, the abdomen was closed without any further procedure. In the stobadine-treated group, stobadine (2 mg/kg) was injected into inferior vena cava before closure of the abdominal wall. Body temperature was maintained close to 37.5°C with a heating lamp. In the I/R and I/R + stobadine-treated groups, both renal arteries were occluded using microvascular clamps for 40 min and occlusion of blood flow was confirmed by visual inspection of the kidneys. After declamping, we confirmed that renal blood flow had been restored prior to closing the incision. At the beginning of reperfusion, stobadine was given to the I/R + stobadine-treated group. The I/R group was not treated. Fluid loss was replaced by the administration of 5 mL warm (37°C) 0.9% NaCl, i.p., prior to abdominal closure in all rats. At the end of 6 h of reperfusion (or the equivalent period of time in the non-I/R rats), 3 mL blood was drawn from the heart and, in the process, the animal was killed. The serum from each animal was isolated to study serum blood urea nitrogen (BUN), creatinine and several other biochemical markers. The abdomen was then re-entered and bilateral nephrectomies were performed. The kidneys were separated into two halves, one half of which was wrapped with aluminium foil, placed in liquid nitrogen and kept at −80°C to determine tissue MDA, GSH and enzyme levels. The other half of each kidney was placed in 10% formaldehyde for histopathological and immunochemical analysis. The same part of each kidney was used consistently for the same determinations in all groups. All specimens were coded in the research laboratory and were evaluated by the same individuals, who were blinded to group assignments.

Biochemical studies

Blood urea nitrogen and creatinine

Serum BUN and creatinine levels were determined with an Abbott-Aeroset autoanalyser (Chicago, IL, USA) using the original kits.

Malondialdehyde and glutathione

Serum levels of MDA (nmol/mL), tissue MDA (nmol/g tissue), serum GSH (nmol/mL) and tissue GSH (μmol/g tissue) were determined spectrophotometrically.[16,17] Tissue (0.5 g) was homogenized using 2 mL ice-cold 10% trichloroacetic acid solution. Then, the homogenate was centrifuged at 1620 *g* for 10 min and the MDA levels in the supernatant assayed by the formation of thiobarbituric acid-reactive substances (TBARS), after which the GSH level was measured. As reported previously,[16,17] 750 μL supernatant was added to an equal volume of 0.67 (w/v) thiobarbituric acid and heated to 100°C for 15 min. The absorbance of the samples was measured at 533 nm. The lipid peroxidation level was expressed in terms of the MDA equivalent using an extinction coefficient of 1.56×105 mol/cm. To determine the GSH levels, 0.5 mL supernatant was added to 2 mL of 0.3 mol/L $Na_2HPO_42H_2O$ solution (pH 7.4). Next, 0.2 mL dithiobisnitrobenzoic acid solution (0.4 mg/mL in 1% sodium citrate) was added and absorbance was measured at 412 nm immediately after mixing. The GSH levels were calculated using an extinction coefficient of 13 600 mol/cm.

Tissue preparation for enzymatic assays

Kidneys were weighed and each sample was homogenized with an ultra turax homogenizer with an S18N-10G probe (Janke and Kunkel, Staufen, Germany) for approximately 3 min, using three volumes of 50 mmol/L potassium phosphate buffer, pH 7.4. The homogenate was centrifuged at 105 000 *g* for 60 min at 4°C using a Beckman ultracentrifuge (Beckman Coulter, Istanbul, Turkey); the resultant supernatants were used for the measurement of enzyme activities.

Chemicals

Stobadine was obtained as a dihydrochloride salt from the Institute of Experimental Pharmacology, Slovak Academy of Sciences (Bratislava, Slovakia). Glucose-6-phosphate (G-6-P), nicotinamide adenine dinucleotide phosphate ($NADP^+$), reduced nicotinamide adenine dinucleotide phosphate (NADPH), 6-phosphogluconate (6-PG), magnesium chloride ($MgCl_2$), oxidized glutathione (GSSG), sodium phosphate monobasic and dibasic, Tris [Tris (hydroxymethyl) aminomethane] were obtained from Sigma Chemical (St Louis, MO, USA). Coomassie brilliant blue G-250 was obtained from SERVA Feinbiochemia (Heidelberg, Germany). Ethanol (EtOH) was obtained from Merck (Darmstadt, Germany). Orthophosphoric acid was obtained from Analar BDH Chemical (Poole, UK).

Enzyme activities

Glucose-6-phosphate dehydrogenase

Enzyme activities were determined spectrophotometrically using an LKB Ultraspec Plus (4054 UV/visible; Cambridge, UK) spectrophotometer, monitoring NADPH production at 340 nm and 37°C.[18] The assay mixture contained 10 mmol/L $MgCl_2$, 0.2 mmol/L $NADP^+$ and 0.6 mmol/L G-6-P in 100 mmol/L Tris/HCl buffer, pH 8.0. Assays were performed in duplicate and activities were followed for 60 s. The reaction was linear during this time. One unit (U) of activity was defined as the amount of enzyme required to

reduce one μmol $NADP^+$/min under the assay conditions. Specific activity was defined as the number of units/mg protein.

6-Phoshogluconate dehydrogenase

6-Phosphogluconate dehydrogenase activity was measured by substituting 0.6 mmol/L 6-PG as substrate in the assay mixture given above for G-6PD measurement.[19]

Glutathione reductase

The activity of GR was measured as described previously.[20] The incubation mixture contained 100 mmol/L sodium phosphate buffer (pH 7.4), 1 mmol/L GSSG, 100 μmol/L NADPH and the tissue supernatants. A decrease in the absorbance of NADPH at 340 nm was monitored spectrophotometrically at 37°C. One unit of activity (U) was defined as the amount of enzyme that catalyses the oxidation of 1 μmol NADPH in 1 min under these conditions.

Glutathione peroxidase

Each 5 μL sample was incubated for 10 min at 37°C in a 495 μL incubation mixture containing 50 μL of 100 mmol/L potassium phosphate buffer (pH 7.0), 5 μL of 100 mmol/L GSH, 10 μL of 200 mmol/L EDTA, 5 μL of 400 mmol/L sodium azide, 50 μL of 2 mmol/L NADPH, 320 μL distilled water and 50 μL GR (10 U/mL).[21] After the 10 min incubation period, the reaction was initiated by the addition of 5 μL of 10 mmol/L H_2O_2. The decrease in the optical density (OD) of the system was measured for 30 s at 340 nm. A similar mixture excluding GSH was used as a blank. One unit of activity (U) was defined the same as for GR.

Protein assay

Protein concentrations were determined according to the methods of Bradford, using bovine serum albumin as a standard.[22]

Histological study

Tissue samples were fixed in a 4% paraformaldehyde solution, embedded in paraffin and 5 μm sections were stained with haematoxylin and eosin. A semiquantitative histological analysis was conducted in a blinded manner. Ten glomeruli and 10 tubules in each kidney were randomly selected at ×400 magnification; damaged glomeruli and tubules were counted and the mean number of damaged glomeruli and tubules was determined. In addition, epithelial and interstitial injuries were noted and graded on a scale of 0–3+ as follows: 0, normal; 1+, slight; 2+, moderate; and 3+, severe. Mean epithelial and interstitial injury scores were determined.

Immunohistochemistry

For the detection of P-selectin, renal tissues were fixed with 10% formalin for 72 h. After fixation, tissues were evaluated using light microscopy. Slices (5 μm) from the paraffin block were stained using a primary antibody (Fitzgerald CD62P, P-selectin, Human/Mouse/Rat; Fitzgerald Industries International, Concord, MA, USA) at room temperature for 1 h. Specimens were viewed under an Olympus BH2 photomicroscope (Olympus, Ankara, Turkey). P-Selectin immunoreactivity was noted and graded on a scale of 0–3+ as follows: 0, normal; 1+, slight; 2+, moderate; and 3+, strong.

Statistical analyses

All statistical analyses were performed using the statistical package SPSS for Windows, version 10 (SPSS, Chicago, IL, USA). All values are expressed as the group mean±SD of determinations for all rats in the group ($n = 10$). Group distributions were analysed by the one-sample Kolmogrov–Smirnov test. All group values exhibited a normal distribution, so that parametric statistical methods were used to analyse the data further. Data were then analysed using analysis of variance (ANOVA) followed by Bonferroni's post test. Histopathological scores, expressed as the mean±SEM, were analysed by the Kolmogorov–Smirnov *Z*-test. $P < 0.05$ was considered statistically significant.

RESULTS

Serum BUN and creatinine

As shown in Fig. 1, renal I/R resulted in significant increases in serum BUN and creatinine levels compared with the sham-operated and stobadine-treated rats ($P < 0.05$). Stobadine administration at the beginning of reperfusion blunted the I/R-induced increases in serum BUN and creatinine levels compared with I/R rats that did not receive stobadine ($P < 0.05$).

Serum and tissue MDA and GSH

Renal I/R produced a significant increase in serum and tissue MDA levels compared with the sham and stobadine-treated groups ($P < 0.05$). Stobadine administration at the beginning of reperfusion significantly reduced serum and tissue MDA levels compared with the I/R group ($P < 0.05$). In addition, renal I/R produced a significant decrease in serum and tissue GSH levels compared with the sham and stobadine-treated groups ($P < 0.05$). Stobadine administration at the beginning of reperfusion significantly prevented the I/R-induced decreases in serum and tissue GSH levels observed in I/R-treated rats. Table 1 gives serum and tissue MDA and GSH levels for the four groups.

Enzyme activities

Renal I/R significantly decreased G-6PD and 6-PGD activities compared with the sham group ($P < 0.05$). Stobadine administration at the

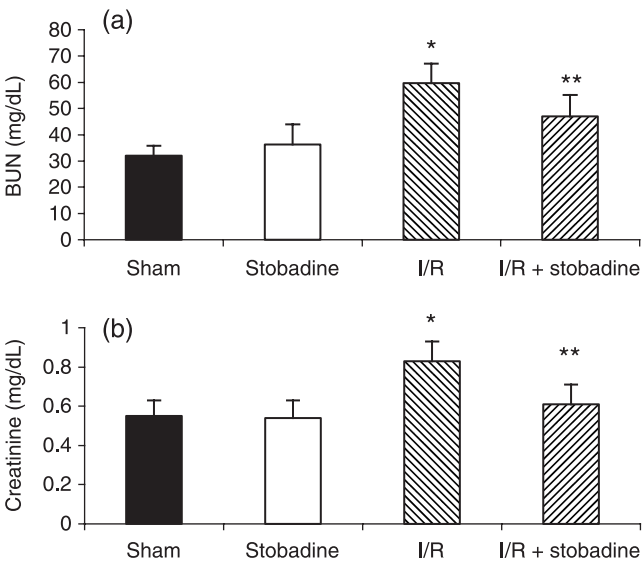

Fig. 1 Rats subjected to ischaemia–reperfusion (I/R) had significantly higher serum (a) bood urea nitrogen (BUN) and (b) creatinine levels than rats that underwent sham operation. Rats that received stobadine at the beginning of reperfusion had significantly lower serum BUN and creatinine levels than rats subjected to I/R alone. Data are the mean±SD. *$P < 0.05$ compared with sham-operated rats; **$P < 0.05$ compared with rats subjected to I/R alone.

Table 1 Serum and tissue malondialdehyde and glutathione levels

	Sham	Stobadine	I/R	I/R + Stobadine
Serum MDA (nmol/mL)	0.04 ± 0.01	0.05 ± 0.01	0.23 ± 0.16*	0.10 ± 0.05
Tissue MDA (nmol/g)	0.06 ± 0.01	0.04 ± 0.01	0.22 ± 0.06*	0.05 ± 0.01
Serum GSH (mmol/mL)	0.68 ± 0.46	0.60 ± 0.04	0.15 ± 0.30*	0.70 ± 0.27
Tissue GSH (μmol/g)	0.44 ± 0.15	0.33 ± 0.04	0.05 ± 0.02*	0.31 ± 0.07†

Values expressed mean±SD ($n = 10$). *$P < 0.05$ for the ischaemia–reperfusion (I/R) group compared with the sham and I/R + Stobadine groups; †$P > 0.05$ compared with the sham group.

MDA, malondialdehyde; GSH, glutathione.

Table 2 6-Phoshogluconate dehydrogenase, glucose-6-phosphate dehydrogenase, glutathione reductase and glutathione peroxidase

Enzymes	Sham	Stobadine	I/R	I/R + Stobadine
G-6PD ($\times 10^{-3}$ U/mg protein)	36.4 ± 7.0*	26.1 ± 2.0	16.5 ± 6.7	25.6 ± 8.9†
6-PGD ($\times 10^{-3}$ U/mg protein)	41.3 ± 6.1*	33.7 ± 7.5	30.5 ± 7.1	32.4 ± 5.1
GR (U/mg protein)	0.3 ± 0.0	0.2 ± 0.0	0.3 ± 0.1	0.3 ± 0.0
GPx (U/mg protein)	1.8 ± 0.2	2.0 ± 0.2	2.0 ± 0.2	2.1 ± 0.4

Values are the mean±SD ($n = 10$). *$P < 0.05$ for the sham group compared wit the ischaemia–reperfusion (I/R) and I/R + Stobadine groups; †$P < 0.05$ compared with the I/R group.

6-PGD, 6-phoshogluconate dehydrogenase; G-6PD, glucose-6-phosphate dehydrogenase; GR, glutathione reductase; GPx, glutathione peroxidase.

beginning of reperfusion significantly ameliorated G-6PD ($P < 0.05$), but not 6-PGD, activity in kidney. Neither GR nor GPx activity was significantly altered in the I/R and I/R + stobadine-treated groups compared with the sham-operated group. All enzyme activities are presented in Table 2.

Histology

Ischaemia–reperfusion injury caused significant glomerular, tubular, epithelial and interstitial damage compared with sham and stobadine treatment ($P < 0.05$). In addition, stobadine administration significantly reduced the morphological damage seen in the I/R group ($P < 0.05$). Figure 2 shows the morphological changes and scores for all groups.

Immunohistochemistry

Kidneys obtained from rats subjected to I/R demonstrated marked staining for P-selectin compared with kidneys obtained from the sham group (Fig. 3a–d), suggesting that this adhesion molecule was expressed during reperfusion. Kidneys obtained from rats treated with stobadine demonstrated markedly reduced staining for P-selectin (Fig. 3e,f) compared with kidneys obtained from I/R groups, suggesting a reduction in the expression of P-selectin during reperfusion.

DISCUSSION

In the present study, we examined the effects of stobadine, a novel synthetic pyridoindole, on renal I/R injury in rats *in vivo*. In addition to functional parameters, morphological changes and P-selectin immunoreactivity demonstrated the protective effects of stobadine against renal I/R injury. The changes in levels of serum and tissue MDA and GSH in all groups suggest that stobadine counteracts at least some of the oxidative stress secondary to I/R.

Acute renal failure is still associated with a relatively high rate of morbidity and mortality. Unfortunately, it is sometimes unavoidable in clinical practice. Ischaemia–reperfusion injury is one of the main causes of acute renal failure and the clinical diagnosis is usually made after the injury has already occurred. Previous studies have demonstrated a promising effect of oral heptanoylstobadine administration against renal I/R injury.[14,15] In these previous studies, heptanoylstobadine was administered 15 days before I/R (to examine its prophylactic effect) or after 60 min ischaemia and 10 min reperfusion (to examine its therapeutic effect). Although the anti-oxidant effects of stobadine in the kidney were demonstrated in these previous studies, no beneficial effects were noted in terms of serum urea and creatinine, which are common physiologial markers of kidney function. Furthermore, serum urea and creatinine were not even examined in one of the studies.

In contrast, in the present study, stobadine (2 mg/kg, i.v.) was injected at the beginning of reperfusion, following 40 min ischaemia. Blood sample collection and tissue harvesting were performed at the end of 6 h reperfusion. Common physiological markers of kidney function (BUN and creatinine) were measured. In addition, we measured the activities of enzyme of the pentose phosphate metabolic pathway and glutathione-dependent mechanisms and performed both histopathological evaluation and P-selectin immunohistochemical staining to allow both a functional and anatomical assessment of the effects of stobadine.

Since it first was developed, in 1983, studies have shown that stobadine has cardioprotective, antihypoxic, anti-arrythmic and neuroprotective effects.[9] The anti-oxidant and ROS-scavenging properties of stobadine are well documented. It is able to scavenge hydroxyl, peroxyl and alkoxyl radicals and quench singlet oxygen.[9,23]

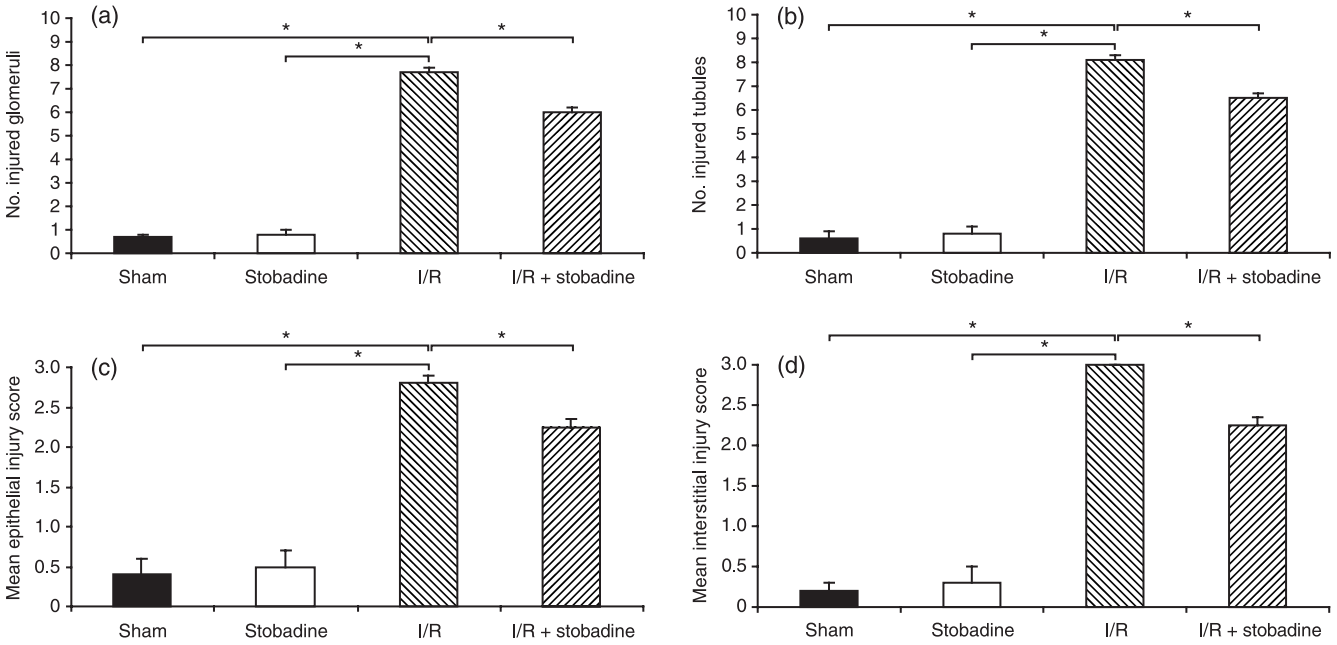

Fig. 2 Scores for morphological change in all groups: (a) glomeruli; (b) tubules; (c) epithelia; and (d) interstitial injury. The increased morphological damage in the ischaemia–reperfusion (I/R) group compared with the sham-operated and stobadine-treated (but no I/R) groups was attenuated by stobadine treatment. Data are the mean±SD. *$P < 0.05$.

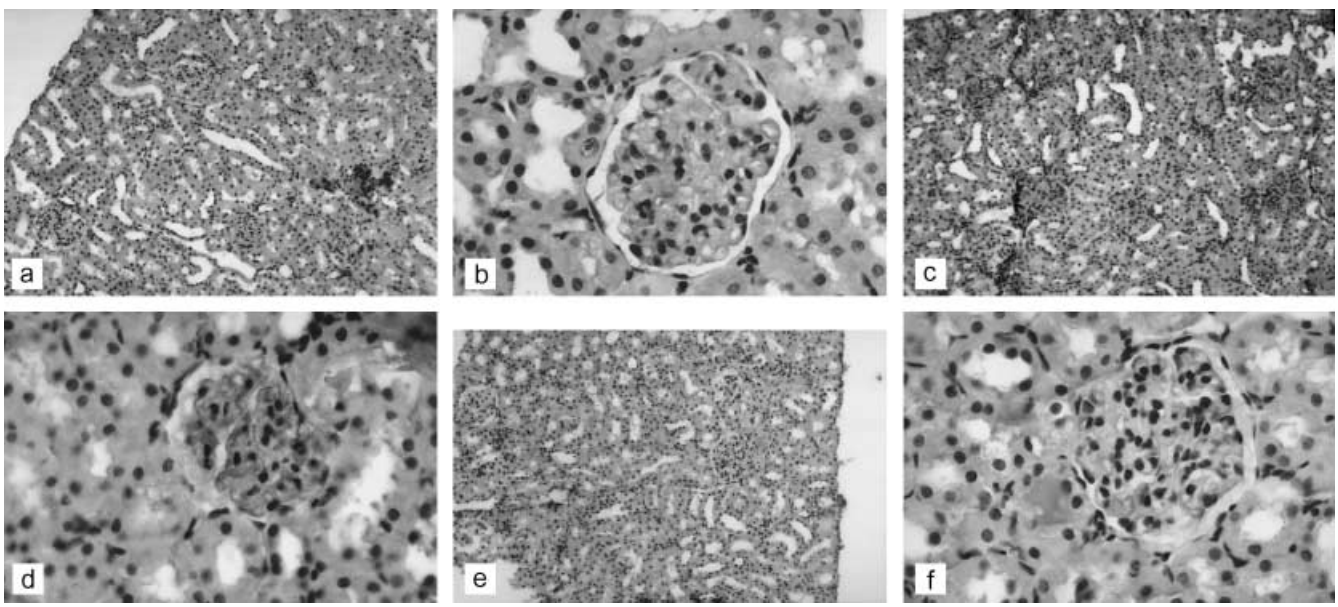

Fig. 3 Immunochemical staining for P-selectin. (a,b) Non-specific immunoreactivity for P-selectin in stobadine-treated rats; (c,d) strong immunoreactivity for P-selectin on capillary walls of a glomerulus in the ischaemia–reperfusion (I/R) group (arrow); (e,f) weak immunoreactivity for P-selectin on a glomerulus in the I/R + stobadine-treated group. (Original magnification ×10 (a,c,e); ×40 (b,d,f)). P-Selectin immunoreactivity was not significantly different between the sham (data not shown) and stobadine (no I/R)-treated groups.

In the present study, stobadine prevented I/R-induced increases in serum and tissue MDA, markers of lipid peroxidation products and lipid peroxidation damage secondary to ROS production.

Glutathione is known as a major defence against oxidative damage.[24] We found both serum and tissue GSH to be decreased after renal I/R injury. However, rats that were treated with stobadine at the beginning of reperfusion exhibited higher GSH levels than rats subjected to I/R alone, which indicates that stobadine helped to replenish the GSH pool. However, neither GR, an important enzyme responsible for maintaining cellular GSH pools, nor GPx significantly changed after I/R. This unexpected finding could be related to the duration of ischaemia or reperfusion, as reported previously.[25,26] In addition, increased tissue GPx and catalase activities reflect increased production of H_2O_2.[27,28] Previous studies using different animal models of oxidative stress have demonstrated that the kidney is more resistant to peroxidative stress than other tissues.[26] Thus, the

present findings regarding glutathione-dependent enzymes after renal I/R may be attributed to differences in the anti-oxidant capacity of the kidney and the severity of the I/R-induced peroxidative stress we administered, as suggested elsewhere.[29,30]

The fundamental enzymes of the pentose phosphate pathway, namely G-6PD and 6-PGD, produce NADPH. It has been reported that when the G-6PD concentration is reduced to 10% of normal, the oxidative pentose phosphate pathway becomes defective, resulting in a reduced content of NADPH.[31] Generation of NADPH is also required for GR activity and GSH production. When NADPH and GSH are deficient, the entrance of oxidizing compounds causes damage to lipids and proteins and consequent cell destruction. Conversely, the requirement for NADH and NADPH as cofactors in the cellular reduction of naturally anti-oxidant compounds has been previously reported.[6,32] Indeed, any change in the activity of G-6PD appears to be very important for the survival of tissues and organisms.[6,32] In the present study, I/R decreased G-6PD and 6-PGD activity in kidney; however, only G-6PD activity was significantly ameliorated with stobadine treatment. We had previously demonstrated in different oxidative stress models that stobadine failed to affect reduced G-6PD and 6-PGD activity in rat brain and aorta.[12] The activity of G-6PD was determined in homogenates of whole cortex and the medulla of rat kidney with unilateral I/R.[33] Thirty minutes of ischaemia decreased G-6PD activity and 60 min reperfusion restored its activity. Nevertheless, G-6PD activity was not determined in the late period. In the present study, however, both renal arteries were occluded and the periods of ischaemia and reperfusion were 40 min and 6 h, respectively. Our results showed that G-6PD activity decreased significantly in the I/R group compared with the sham group. Conversely, both the preservation of renal function, evidenced by attenuation of serum BUN and creatinine and the partial prevention of kidney tissue histopathology, suggests that stobadine has a protective effect against I/R-induced tissue injury. In addition, the observed beneficial effects of stobadine may be linked to its effects on tissue Ca^{2+}-ATPase activity and calcium metabolism.[34]

Finally, in the present study, we found that stobadine decreases the high P-selectin immunoreactivity observed in renal tissue after I/R. P-Selectin, an adhesion molecule, is stored in endothelial cells and platelets. The selectin family is responsible for the first step of inflammation and can be detected, after activation, on the cell surface. P-Selectin immunoreactivity has been used in animal and human clinical studies as a marker of injury.[35–37] In mice, it has been shown that P-selectin expression begins at 20 min of renal reperfusion following 30 min ischaemia, peaking at 5 h and then declining at 10 h.[37] Although P-selectin activity was not detectable in normal glomeruli by immunostaining, increased P-selectin immunoreactivity was abundant in glomeruli subjected to I/R in the present study. However, stobadine decreased the high P-selectin immunoreactivity otherwise seen post-I/R.

In conclusion, 40 min ischaemia followed by 6 h reperfusion caused renal dysfunction, oxidative damage, morphological injury and high P-selectin immunoreactivity in the animal model used in the present study. However, i.v. administration of stobadine at the beginning of reperfusion blunted I/R-induced increases in serum BUN and creatinine, prevented both serum and tissue MDA increases and maintained serum and tissue GSH levels at normal levels. In addition, stobadine prevented morphological damage and decreased the high P-selectin immunoreactivity otherwise observed secondary to I/R injury.

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