Intrarenal administration of recombinant human soluble thrombomodulin ameliorates ischaemic acute renal failure

Takenori Ozaki1, Chabouk Anas1,2, Shoichi Maruyama1, Tokunori Yamamoto2, Kaoru Yasuda1, Yoshiki Morita1, Yasuhiro Ito1, Momokazu Gotoh2, Yukio Yuzawa1 and Seiichi Matsuo1

1Department of Nephrology and 2Department of Urology, Nagoya University Graduate School of Medicine, 65 Tsurumaicho, Showaku, Nagoya, Japan 466-8550, Japan

Abstract

Background. Thrombomodulin (TM) is an endothelial anti-coagulant cofactor which also has anti-inflammatory properties. The present study was performed to investigate the effects of recombinant human soluble TM (RHS-TM) on ischaemia/reperfusion (I/R) renal injury.

Methods. A right nephrectomy was performed in rats, and the left kidney was filled with RHS-TM (0.25 mg/kg), argatroban (20 mg/kg) or a vehicle for 45 min. Before reperfusion, the fluid trapped in the kidney was completely removed. At 24 h after I/R, renal cortical blood flow was measured using a CCD video camera, and the kidneys were harvested for the study. Next, cultured human umbilical vein endothelial cells were treated with RHS-TM (2, 10 or 50 mg/ml) or a vehicle, and incubated for 5 h in culture medium containing 300 μM hydrogen peroxide. Apoptotic cell death was analysed by terminal deoxynucleotidyl transferase dUTP nick-end labelling (TUNEL) assay.

Results. Immunohistochemistry revealed that the level of TM expression decreased in rat kidneys after I/R. RHS-TM significantly decreased blood urea nitrogen and serum creatinine levels. It also prevented a reduction in cortical blood flow, and attenuated tubular damage and macrophage/neutrophil infiltration. In addition, the number of TUNEL-positive cells decreased significantly in rats treated with RHS-TM.

Conclusion. The transient intrarenal administration of RHS-TM, but not argatroban, to the kidney attenuates I/R renal injury. The present study suggests that RHS-TM would be a useful tool in preventing transplanted kidney damage or treating acute renal failure in the clinical setting.

Keywords: apoptosis; blood flow; endothelium; ischaemia; thrombomodulin

Introduction

Acute renal failure is a major clinical problem and the morbidity and mortality of affected patients remain high [1]. Ischaemia/reperfusion (I/R)-induced renal injury is an important cause of acute renal failure [2]. It occurs when renal perfusion is reduced during shock. It is also a significant complication of vascular surgery of the aorta or kidney [3]. Furthermore, I/R injury occurs in the allografts during the process of organ retrieval, storage and re-establishment of blood flow. It is a common cause of impaired or delayed graft function [4]. I/R injury is also known to be closely associated with increased acute rejection episodes and late allograft failure [5]. These problems are almost always observed in cadaveric renal allografts that have been subjected to warm or cold ischaemia, then reperfusion [4]. The unsatisfactory results from such transplants hamper efforts to enlarge the donor pool through the use of marginal donor organs [6]. Therefore, efforts to reduce damage to kidneys exposed to I/R-induced injury are essential.

Thrombomodulin (TM) is a widely expressed endothelial cell membrane-bound glycoprotein, which is known to form a high-affinity complex with thrombin and converts thrombin from a procoagulant to an anti-coagulant enzyme [7]. The thrombin–TM complex potently activates anti-coagulant protease protein C, thereby inhibiting thrombus formation [7,8]. TM is known to preserve the integrity of the endothelium. When endothelial cells are damaged, soluble TM is released into circulation [9,10]. Sido et al. [11] reported that the levels of soluble TM in the sera of patients who received liver transplant correlated...
well with the degree of early graft damage. Soluble TM can be used as a marker of endothelial cell damage. Conversely, Salomaa et al. [12] demonstrated that a high plasma level of soluble TM in healthy people may protect the endothelial cells from injury and is associated with decreased risk of coronary heart disease.

Recombinant human TM (RHS-TM), a soluble derivative of human TM, has been produced [8]. Like native TM, RHS-TM binds directly to thrombin, and shows potent anti-coagulant activity [13]. RHS-TM is effective in animal models of disseminated intravascular coagulation [14–16]. Moreover, recent studies have shown that RHS-TM acts as a potent anti-inflammatory molecule in settings such as endotoxin-induced tissue damage [17] and atherothrombosis [18]. RHS-TM is also known to reduce liver damage following hepatectomy in cirrhotic rats, and suppresses I/R injury of the liver [19]. Concerning the kidney and RHS-TM, we have demonstrated that RHS-TM suppresses leucocyte/macrophage infiltration in the glomeruli and attenuated the renal damage in a rat model of thrombotic glomerulonephritis [20].

In our efforts to find a better way to protect the kidney from I/R injury, we hypothesized that soluble TM would be released from the endothelial cells of the kidney after I/R, and that the local administration of RHS-TM would ameliorate the renal injury by suppressing inflammation as well as by attenuating apoptosis. The purposes of the present study were to determine the therapeutic effects of RHS-TM on I/R renal injury and investigate the possible mechanisms involved.

Materials and methods

Animals

Male Sprague-Dawley rats weighing 250–300 g were purchased from Chubu Kagaku Shizai Co. Ltd (Nagoya, Japan) and were allowed free access to food and water. The protocols were approved by the Animal Care and Use Committee of Nagoya University, and the experiments were performed according to the Animal Experimentation Guidelines of Nagoya University Graduate School of Medicine.

Experimental protocols

Experiment 1

Six rats were anaesthetized by the intraperitoneal injection of sodium pentobarbital (50 mg/kg body weight) and a right nephrectomy was performed via flank incision. Seven days later, rats were anaesthetized again by the intraperitoneal injection of sodium pentobarbital, and the abdominal cavity was opened via midline incision. The viscera were placed aside and wrapped with wet gauze, the aorta and renal vessels (artery and veins) were exposed and isolated. The aorta was clamped above the left renal artery first and then below. A 24-gauge cannula was placed in the aorta between the two clamps, and 2 ml of saline was administered slowly into the left kidney. Then, the renal artery and vein were clamped, the hole was covered with surgical glue and the original clamps were removed. Blood flow was restored after 45 min of ischaemia. The left kidneys were removed at 5 min (n = 3) and 18 h (n = 3) after reperfusion. Kidneys from three other normal rats served as controls.

Experiment 2

A right nephrectomy was performed in 24 rats. Seven days later, I/R renal injury was induced in a similar way as in Experiment 1. The left renal vein was clamped and a hole was made in the vein wall using a 23-gauge needle and RHS-TM (0.25 mg/kg) dissolved in 2 ml of saline (n = 6), argatroban (20 mg/kg) in 2 ml of saline (n = 6), or 2 ml saline only (n = 6) was injected slowly into the left kidney, forcing the blood out through the hole in the vein, until all the blood in the kidney had been drained. In order to ensure comparable anti-coagulant activities (elongation of APTT), appropriate dosages of each drug were determined based on information from a previous study [13]. All excess RHS-TM, argatroban, or saline which exited the vein hole was completely absorbed using a sponge on a stick. The renal artery and vein were clamped with a 10 mm micro-aneurysm clip (Mizuho Ikakogyo, Tokyo, Japan). The aorta hole was closed with surgical glue, and the two aorta clips were taken off, leaving the injected fluid trapped inside the kidney. After 45 min of ischaemia, another clamp was placed at the renal vein and the clip at the renal artery and vein was removed. The RHS-TM, argatroban, or saline trapped in the kidney during the ischaemic period came out of the vein hole, and the fluid was again completely wiped away. Then, the hole was closed by applying pressure for 5 min. Control rats (n = 6) underwent sham operation. At 24 h after reperfusion, RBC velocity in peritubular capillaries was measured using a pencil type CCD video microscope [21]. Rats were then sacrificed and blood and kidney samples were collected for the study. Normal rats (n = 4) were also used to obtain the baseline data of renal cortical microcirculation.

Direct vision using a CCD video microscope

An intravital CCD video microscope which has a pencil lens probe with a tip diameter of 1 mm was used in this study. The probe has a magnification of ×520 and spatial resolution of 0.86 μm, permitting identification of individual blood cells [21,22]. This device enabled us to get a direct image of microcirculation in various organs in humans as well as organs in large and small animals, including pigs, rabbits, rats and mice. The blood flow in renal CTC was observed, and the images were recorded using the freeze-frame mode.
The speed of RBCs in individual segments of the CTC was analysed using a point tracking method and motion detection program.

**Coagulation parameters, serum creatinine and blood urea nitrogen**

Two millilitre of blood was transferred into a plastic syringe containing 0.22 ml of 3.8% trisodium citrate, and plasma was prepared by centrifugation at 1710 g for 10 min. Prothrombin time (PT) and activated partial prothrombin time (APTT) were measured by Mitsubishi BCL Co. Ltd (Tokyo, Japan). The rest of the blood was collected into a glass tube, stored overnight and centrifuged at 1500 r.p.m. for 10 min. Serum creatinine and blood urea nitrogen (BUN) were measured using Daiya Auto UN or Daiya Auto Crea Kit (Daiya Shiyaku, Tokyo, Japan).

**Histology and immunohistochemistry**

One part of the kidney was fixed with 4% paraformaldehyde, embedded in paraffin, and cut into 4 μm sections. These were stained with haematoxylin and eosin (HE) and periodic acid-Schiff reagent (PAS). Another part of the kidney was frozen in OCT compound (Miles, Elkhart, IN). Sections (2 μm thick) were fixed in acetone. Immunostaining was performed as previously described [23]. TM was stained with rabbit anti-rat TM Ab [24], followed by a fluorescein conjugate of goat anti-rabbit IgG Ab (Zymed Laboratories, San Francisco, CA). Monocytes/macrophages were stained with a fluorescein conjugate of mouse anti-rabbit ED1 Ab (Serotec, Raleigh, NC).

**Morphometric analysis of histology and immunohistochemistry**

To assess tubulointerstitial injury, PAS stained kidney sections were analysed using a semiquantitative grading as described previously [25] with minor modification. Briefly, the extent of tubular cast formation and tubular damage (dilatation and degeneration) in 16 non-overlapping fields (×200) of the outer medulla was scored according to the following criteria: 0, normal; 1, less than 10%; 2, 11–25%; 3, 26–45%; 4, 46–75% and 5, more than 76% of the pertinent area. Macrophage infiltration was assessed by counting the ED-1-positive cells in 16 non-overlapping fields (×400) of the outer medulla on frozen sections, and the numbers were expressed per field. Neutrophil infiltration was also evaluated using the HE sections in a similar way. The morphologic analysis was carried out by two observers in a blind fashion, using a Zeiss microscope (Oberkochen, Germany).

To evaluate the vascular density, kidney sections stained for rat TM were analysed. Photographs of six non-overlapping fields (×200) in the outer medulla were taken, and the areas positively stained for rat TM were measured using the MetaMorph 6.3 image analysis computer program (Universal Imaging Co., West Chester, PA) [26].

**Experiment 3**

Dose dependency was studied using the same protocol as in Experiment 2. Right nephrectomy was performed on 20 rats. Seven days later, 2 ml of saline, or two different doses (0.025 and 0.25 mg/kg) of RHS-TM dissolved in 2 ml of saline (each n = 5) was injected into the left kidney. After 45 min of ischaemia, all kidneys were completely cleared of the administered reagents. Five rats were subjected to sham operation. At 24 h, rats were sacrificed and blood samples were collected for the study.

**In vitro experimental design**

Human umbilical vein endothelial cells (HUVECs) (Takara, Ootsu, Japan) were cultured in endothelial cell basal medium-2 (EBM-2) supplemented with 100 U/ml penicillin, 100 μg/ml streptomycin and an EGM-2 using the EGM-2 BulletKit (Takara). HUVECs were grown up to 90–95% confluence on four-well chamber slides (Nalge Nunc International, Rochester, NY), and cytoprotective effects of THS-TM were studied as described [27]. RHS-TM in doses of 2, 10 or 50 mg/ml, or saline was added to HUVECs in culture, and then incubated with 300 μM hydrogen peroxide for 5 h. Following incubation, apoptotic cell death was analysed by terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick-end labelling (TUNEL) assay.

**TUNEL assay**

Apoptotic cell death was determined on paraffin-embedded kidney sections (4 μm-thick) or HUVECs fixed in 4% paraformaldehyde using in situ Apoptosis Detection Kit (Takara) as described [27]. Six non-overlapping fields (×200) in the outer medulla of the kidney sections, and six non-overlapping fields (×200) on the cell culture chamber slides were photographed. The numbers of TUNEL-positive cells were counted using MetaMorph 6.3 (Universal Imaging Co.).

**Statistical analyses**

Statistical analysis was performed using a software program, Stat View 5.0 (SAS Institute, Cary, NC, USA). Two-way analysis of variance (ANOVA) was used to determine the significant difference among three groups. When statistical difference was indicated by ANOVA, further analysis was performed using Scheffé to determine the difference between any pair of groups. A significant difference was defined as a P-value of <0.05. All values are provided as mean ± SD.
Results

Experiment 1

Effects of Ischaemia on the TM of the endothelium. In the normal rat kidney, TM was stained strongly on the peritubular capillaries. At 5 min after reperfusion, TM expressed in a patchy pattern with only weak staining in some parts of the peritubular capillaries especially in the outer medulla of the kidney. At 18 h after reperfusion, the pattern of TM expression appeared irregular, and the intensity of the staining greatly diminished (Figure 1).

Experiment 2

Effects of RHS-TM on renal function and coagulation parameters. Rats which underwent renal I/R exhibited significant increases in levels of serum creatinine and BUN (P < 0.01 vs the sham group). RHS-TM significantly suppressed these increases (P < 0.05 vs the saline group). In the argatroban group, however, serum creatinine and BUN levels showed a tendency to be lower, but not statistically significant (Figure 2). Significant differences were not observed in PT or APTT among four groups (Figure 2).

Effects of RHS-TM on renal cortical microcirculation and tubules. By direct imaging of the renal cortex at 24 h after IRI using a CCD video microscope, information on renal blood flow and the condition of the renal tubules was obtained (Figure 3). The movement of the erythrocytes was observed in the cortical peritubular capillaries and RBC velocity in the cortex was determined by the analysis of the speed of the erythrocytes. Gross differences were observed between the saline-injected rats and normal rats. In the saline group, the renal cortical peritubular capillaries shrunk and blood flow was severely impeded as compared with normal rats; the speed was 0.15 ± 0.02 mm/s in the saline group and 0.59 ± 0.08 mm/s in normal rats, P < 0.01. Furthermore, most of the renal tubules were destroyed or obstructed by detached epithelial cells. In contrast, in RHS-TM-injected rats, the condition of the cortical capillaries was close to that of normal rats. Only a mild destruction of the tubules was observed, and RBC velocity in the cortex significantly improved: the speed was 0.35 ± 0.07 mm/s in the RHS-TM group (P < 0.01 vs the saline group). In contrast, among argatroban-treated rats, severe destruction and obstruction of the tubules was observed, and RBC velocity was not significantly different from the saline-treated rats (Figure 3).

Effects of RHS-TM on tubular injury. Examination of PAS stained kidney sections from the saline-treated rats showed that tubular injury occurred mainly in the outer medulla of the kidney. RHS-TM greatly ameliorated the tubular injury, while argatroban partially attenuated the tubular damage (Figure 4). The severity of the tubular damage, including tubular dilatation, degeneration and cast formation was scored. Treatment with RHS-TM resulted in significantly better scores than the saline group (2.2 ± 1.5 vs 4.4 ± 0.5, P < 0.01). In contrast, the argatroban-treated group (2.9 ± 1.2) failed to show significantly better scores than the saline-treated group (Figure 4).

Effects of RHS-TM on cellular infiltration. The numbers of macrophages decreased in both the RHS-TM and argatroban groups compared with the saline group (3.25 ± 1.04, 4.10 ± 0.56, 2.04, and 7.08 ± 1.71, respectively, P < 0.01) (Figure 5). RHS-TM administration also suppressed neutrophil infiltration when compared with saline (2.07 ± 0.64 and 5.83 ± 2.04, respectively, P < 0.01). In contrast, administration of argatroban (3.82 ± 1.61) did not make a significant difference (Figure 6).

Effects of RHS-TM on apoptosis in the kidney. The contribution of apoptosis to the renoprotective properties of RHS-TM was assessed by performing TUNEL assay on the kidney sections. In the saline-treated rats, TUNEL-positive cells were localized mainly in the tubules of outer medulla. RHS-TM significantly decreased the number of TUNEL-positive cells as compared with saline only (8.52 ± 4.6 and 26.4 ± 7.4, P < 0.05) while argatroban did not (20.26 ± 10.5) (Figure 6). Only a few TUNEL-positive cells were observed in the sham group.

Effects of RHS-TM on the expression of rat TM. Peritubular capillaries were strongly stained for rat TM in the sham group, whereas, only weak and patchy staining for rat TM was observed on the peritubular capillaries of the outer medulla at 24 h after reperfusion in the saline group—consistent with the results shown in Experiment 1. RHS-TM significantly
prevented the reduction of TM expression on the endothelium, while argatroban did not (Figure 7).

Experiment 3

*Dose-dependent effects of RHS-TM.* Rats given saline only exhibited significant increases in levels of serum creatinine at 24 h after renal I/R. RHS-TM significantly suppressed the increase when given in a dose of 0.25 mg/kg. A lower dose (0.025 mg/kg) of RHS-TM also showed a tendency to improve the renal function, which, however, was not statistically significant (Figure 8).

**In vitro experiment**

*Effects of RHS-TM and argatroban on apoptosis in HUVECs.* In order to study the endothelial cell damage by oxidative stress, HUVECs were incubated in culture medium containing 300 μM hydrogen peroxide and one of three different doses of RHS-TM (2, 10 or 50 μg/ml), or saline. A significant number of TUNEL-positive cells were observed in HUVECs treated with hydrogen peroxide and saline. RHS-TM attenuated the number of TUNEL-positive cells induced by hydrogen peroxide in a dose-dependent manner (Figure 9).

**Discussion**

The present study demonstrated that the levels of TM expression in the kidney decreased after ischaemia and reperfusion. It is known that plasma concentrations of soluble TM are elevated in critically ill patients, such as those with disseminated intravascular coagulation or sepsis [9,10]. This is most likely due to endothelial damage with increased release of membrane-bound TM into the circulating blood. It has been reported that TNF-α causes the endothelial cells to release TM from their surface [28]. Moreover, it was demonstrated that soluble TM was released from the liver graft into circulation, and those levels correlated well with early liver damage observed among patients who received...
liver transplant [11]. In our study as well, it is likely that the extracellular part of TM was released from the endothelial cells damaged by the ischaemia. Therefore, it would be reasonable to assume administration of RHS-TM ameliorates ischaemic renal damage.

We studied the therapeutic effects of RHS-TM using a rat model of I/R injury. The results clearly demonstrated that intrarenal administration of RHS-TM before reperfusion markedly ameliorated renal function and tubular damage. Renal cortical microcirculation was also significantly improved by RHS-TM, suggesting that endothelial cell dysfunction [22] may be attenuated by RHS-TM. The protocol, transient intrarenal administration of RHS-TM, was employed in this study in order to mimic the situation of kidney transplantation, where RHS-TM can be applied to the donor kidney during the harvesting period [29]. Because anti-coagulatory effects of RHS-TM may be harmful for the recipient during the operation, the protocol was made to fully prevent the systemic action of RHS-TM. This protocol may also implicate the possible application of RHS-TM to the kidney during warm ischaemic period in the major vascular surgery.

In the present study, argatroban, a direct thrombin inhibitor, was used as a control reagent. RHS-TM reduced macrophage and granulocyte infiltration, and suppressed apoptotic cell death in the kidney.

Fig. 3. Effects of RHS-TM on renal cortical microcirculation. Representative photographs show the images of the renal cortical microcirculation recorded using a CCD video microscope. In the saline group (upper right panel), the renal cortical peritubular capillaries shrunk as compared with normal rats (upper left panel). The condition of the cortical capillaries in the RHS-TM group (lower left panel) was close to that of normal rats. In contrast, the appearance of the peritubular capillaries of the argatroban group (lower right panel) was not different from that of the saline group. Graphic presentation demonstrates that RBC velocity was severely impeded after renal ischaemia and that RHS-TM significantly improved RBC velocity, while argatroban did not. Arrow heads indicate the vessels measured. **P < 0.01. Bar, 100 µm.

Fig. 4. Histology of the kidneys. Representative micrographs show renal histology (PAS). The sham-operated rats showed normal histology (upper left panel). The saline-treated rats (upper right panel) show tubular cast formation, tubular dilatation and tubular degeneration in the outer medulla of the kidney. Kidney sections from rats treated with RHS-TM (lower left panel) show only mild tubular damage. Argatroban partially attenuates the tubular damage (lower right panel). Graphic presentation demonstrates the scores indicating the severity of the tubular damage. The RHS-TM group showed significantly better scores than the saline group, while the argatroban group failed. *P < 0.05. Bar, 100 µm.
Argatroban significantly suppressed the number of macrophages, but not granulocytes. It also showed a tendency to ameliorate renal function; this however was not statistically significant. It is known that coagulation and inflammation enhance each other, leading to tissue damage [20]. The moderate therapeutic effects of argatroban can be explained by its anti-coagulatory properties. The results of the present study suggest that RHS-TM has some effect beyond that of an anti-coagulant.

There are several possible pathways by which RHS-TM exerts its renoprotective properties. First, RHS-TM can suppress inflammation by inhibiting the activation of proteinase-activated receptor-1 (PAR-1, one of the thrombin receptors). Thrombin is known to promote inflammatory reactions by activating PAR-1, which is reported to be involved in the various processes of renal injury [30,31]. Although histology did not show thrombus formation in the control kidneys after I/R injury, it is possible that a small amount of thrombin was generated locally in the renal microcapillaries, resulting in the activation of PAR-1. In the present study, the number of macrophages decreased when either RHS-TM or argatroban was administered, suggesting that the effect of RHS-TM on macrophage infiltration was attributable to its direct anti-thrombin action. Nevertheless, the fact that argatroban failed to ameliorate renal function demonstrates that the inhibition of PAR-1 alone was not
sufficient to effectively suppress I/R injury. Secondly, TM can strongly enhance thrombin’s ability to activate protein C (ikeguchi 42–50). Activated protein C is known to be a potent anti-inflammatory molecule. In fact, previous studies demonstrated that activated protein C attenuates I/R injury of the liver [32] and the kidney [33], and that heparin did not show renoprotection [33]. Still, it is not likely that RHS-TM acted through protein C in the present study, since RHS-TM can efficiently activate human protein C but not protein C of rat origin [34]. Thirdly, RHS-TM may exert its anti-inflammatory effects by activating proCPR (or TAFI). In a previous study, we demonstrated that RHS-TM suppresses leucocyte/macrophage infiltration in the glomeruli of rats with thrombotic glomerulonephritis [20]. When RHS-TM binds to thrombin, the complex potently activates proCPR to CPR, which degrades the anaphylatoxins C3a and C5a by cleaving their terminal arginine residues [35]. Again, it is not very likely that RHS-TM enhanced the action of anaphylatoxins via activating proCPR in this study since thrombus formation or complement deposition was not evident in the glomeruli after I/R injury, whereas massive thrombosis and strong C3 deposition were observed in the glomeruli of rats with thrombotic glomerulonephritis as shown in our previous study [20].

Recently, more direct anti-inflammatory effects of TM have also been demonstrated. RHS-TM is composed of three domains: an N-terminal lectin-like domain, an EGF-like domain consisting of six EGF-like repeats and a Ser/Thr–rich domain [8,36]. An EGF-like domain is critical for the anti-coagulant cofactor activities of TM (i.e. inhibition of thrombin and promotion of APC formation) [8,37]. It was shown that the N-terminal lectin-like domain of TM confers protection from inflammatory cell mediated tissue damage by suppressing adhesion molecule expression via NF-κB and mitogen-activated protein kinase pathways [38]. Moreover, Abeyama et al. [39] reported that the N-terminal lectin-like domain of TM acts against inflammation by sequestering high-mobility group-B1 protein. Therefore, the anti-inflammatory property of RHS-TM may be attributable to these direct effects of TM.

Immunostaining for rat TM showed that RHS-TM attenuated the reduction in the level of rat TM at 24 h after I/R injury, suggesting that RHS-TM may have exerted renoprotection by limiting endothelial cell injury. In order to study the effects of RHS-TM on the endothelial cells, apoptotic cell death was examined by in vitro experiments using HUVECs. TUNEL assay demonstrated that hydrogen peroxide induced a substantial degree of apoptosis, and the number of apoptotic cells was significantly attenuated by
incubation with RHS-TM. Conway et al. [38] reported that the soluble lectin-like domain of TM protects vascular endothelial cells from serum deprivation–induced cell death. The result was consistent with our data. Conway and Abeyama also showed that the lectin-like domain of TM suppresses NF-κB activation [39]. NF-κB activation is shown to play a role in the process of apoptosis induced by reactive oxygen intermediates [40]. Therefore, suppression of NF-κB could be one of the mechanisms by which RHS-TM protected endothelial cells from apoptosis. However, other studies showed that certain kinds of lectins inhibit apoptotic cell death via different pathways [41,42]. Further studies will be needed to explore the anti-apoptotic effects of RHS-TM.

In conclusion, the results of our study provide evidence that the transient intrarenal administration of RHS-TM during the warm ischaemic period attenuates renal injury. The findings may have therapeutic implications for the preservation of transplanted kidneys or in preventing acute renal failure after major vascular surgery.

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Conflict of interest statement. None declared.

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