Preventive Effect of SA13353 [1-[2-(1-Adamantyl)ethyl]-1-pentyl-3-[3-(4-pyridyl)propyl]urea], a Novel Transient Receptor Potential Vanilloid 1 Agonist, on Ischemia/Reperfusion-Induced Renal Injury in Rats

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ABSTRACT

Tumor necrosis factor (TNF)-α plays a crucial role in the pathogenesis of ischemia/reperfusion-induced renal injury. We have demonstrated recently that the preischemic treatment with resiniferatoxin, a transient receptor potential vanilloid 1 (TRPV1) agonist, attenuates renal TNF-α mRNA expression and improves ischemia/reperfusion-induced renal injury. In addition, we found that SA13353 [1-[2-(1adamantyl)ethyl]-1-pentyl-3-[3-(4-pyridyl)propyl]urea], a novel transient receptor potential vanilloid 1 (TRPV1) agonist, inhibits TNF-α production through the activation of capsaicin-sensitive afferent neurons and reduces the severity of symptoms in established rat collagen-induced arthritis. In the present study, we investigated effects of treatment with SA13353 on ischemia/reperfusion-induced renal injury in rats. Ischemic acute kidney injury (AKI) was induced by occlusion of the left renal artery for 45 min followed by reperfusion. Two weeks after contralateral nephrectomy. Renal function, renal injury, neutrophil infiltration, superoxide production, TNF-α mRNA expression, and cytokine-induced neutrophil chemoattractant-1 mRNA expression were augmented, but these alterations were attenuated by treatment with SA13353. On the other hand, ischemic acute kidney injury was exacerbated in SA13353 treatment. These results demonstrate that the capsaicin-sensitizing agent SA13353 prevents the ischemia/reperfusion-induced AKI. This renoprotective effects seem to be closely related to the inhibition of inflammatory response via TRPV1 activation.

Renal ischemia, followed by reperfusion, is one of the most common causes of acute kidney injury (AKI) and places a significant burden on the health care system. AKI is caused by ischemic and nephrotoxic insults acting alone or in combination. It is associated with increased morbidity, prolonged hospitalizations, and increased mortality (Kelly and Moliterno, 2000). The renal tubules are susceptible to hypoxic injury because of a number of factors, but they are also capable of rapid regeneration and functional recovery. The molecular mechanisms underlying ischemia/reperfusion-induced renal injury are not fully understood, but it has been reported that several causal factors [e.g., tumor necrosis factor (TNF)-α mRNA expression, ATP depletion, enhancement of reactive oxygen species production, phospholipase activation, neutrophil infiltration, vasoactive peptides, etc.] are contributive to the pathogenesis of this renal damage (Edelstein et al., 2000).

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ABBREVIATIONS: AKI, acute kidney injury; TNF, tumor necrosis factor; IL, interleukin; TRPV1, transient receptor potential vanilloid type 1; SA13353, 1-[2-(1-adamantyl)ethyl]-1-pentyl-3-[3-(4-pyridyl)propyl]urea; CINC, cytokine-induced neutrophil chemoattractant; BUN, blood urea nitrogen; Pcr, plasma creatinine concentration; FE_{urea}, fractional excretion of sodium; PCR, polymerase chain reaction; Rps18, ribosomal protein S18; CGRP, calcitonin gene-related peptide; LPS, lipopolysaccharide.
Among them, TNF-α is released during renal ischemia/reperfusion and plays an important role in the ensuing neutrophil-mediated kidney injury (Donnahoo et al., 1999). TNF-α up-regulates neutrophil adhesion molecules, in particular intercellular adhesion molecule-1, after renal ischemia/reperfusion, and this molecule then plays an important role in tissue neutrophil influx. Accumulating evidence indicates that renal TNF-α is an autocrine contributor to renal dysfunction induced by the ischemia/reperfusion (Ramesh and Reeves, 2004). On the other hand, the anti-inflammatory cytokine interleukin (IL)-10, which is a potent inhibitor of fever generation and early phase inflammation, has many effects such as inhibition of cytokines, chemokines, and neutrophil activation (de Vries, 1995). In addition, IL-10 has been shown recently to improve ischemic and cisplatin-induced AKI by inhibiting the maladaptive activation of genes that cause leukocyte activation and adhesion (Deng et al., 2001).

Transient receptor potential vanilloid 1 (TRPV1), formerly termed vanilloid receptor 1, is a nonselective cation channel that can be activated by noxious heat, protons, and vanilloids, such as capsaicin (Caterina et al., 1997), and is a range of putative endogenous mediators (Van Der Stelt and Di Marzo, 2004). TRPV1 was known originally to be a receptor with nociceptive primary afferent fibers, but subsequent observations revealed that it is broadly expressed in the epidermis, and visceral cells (Nagy et al., 2004). It is controversial that TRPV1 agonists act as aversive or inflammatory or inhibitors of it. Keeble et al. (2005) and Szabó et al. (2001) have demonstrated that TRPV1 agonists have a potent role in acute and chronic inflammation in the knee joint. In contrast, Kissin et al. (2005) have shown that capsaicin, a potent capsaicin analog, can activate TRPV1 in the kidney. Small doses of capsaicin are known to reduce systemic inflammatory responses in septic rats (Mirkible et al., 2004). Moreover, it has been demonstrated that lipo-polysaccharide-induced systemic endotoxemia and airway inflammation are augmented in TRPV1 knockout mice, thereby suggesting the protective role of TRPV1-mediated action against the onset of lung edema and endotoxin-induced airway inflammation (Cowley et al., 2004; Helyes et al., 2007). A recent study demonstrated that the recovery process of ischemia/reperfusion-induced cardiac dysfunction was impaired in TRPV1 gene knockout mice (Wang and Wang, 2005).

TRPV1 expression is seen in a high percentage of sensory afferent neurons that project to cardiovascular and renal tissues (Wang, 2005). It has been demonstrated most recently that TRPV1 protein is highly present in the renal pelvis and exclusively regulates neuropoietic release from primary renal afferent neurons in response to mechanostimulation (Feng et al., 2008). Moreover, TRPV1 protein is abundantly located in renal tubules of medulla, although its functional roles are unknown (Feng et al., 2008). The renal medulla contains nephrons that are most susceptible to ischemic injury (Brady et al., 2000). We have obtained evidence recently that the TRPV1 agonist capsaicin and resiniferatoxin attenuate renal TNF-α mRNA, increase renal IL-10 mRNA expression, and improve ischemia/reperfusion-induced renal injury in rats (Ueda et al., 2008). SA13353 was originally identified by us as a potent and orally active inhibitor of TNF-α production, and this effect was due to the activation of capsaicin-sensitive afferent neurons mediated via TRPV1 in vivo (Murai et al., 2008). In the present study, we investigated the effects of treatment with the TRPV1 agonist, SA13353, on the ischemic AKI in rats and evaluated the possible involvement of TNF-α, IL-10, and cytokine-induced neutrophil chemoattractant (CINC) in the SA13353-induced actions.
weeks before the study (at 8 weeks of age), the right kidney was removed through a small flank incision under pentobarbital anesthesia (50 mg/kg i.p.). After a 2-week recovery period, uninephrectomized rats were divided into sham-operated control, vehicle-treated ischemic AKI, and drug-treated ischemic AKI groups. These all animals abstained from food for 12 h before the ischemia. To induce ischemic AKI, the rats were anesthetized with pentobarbital (50 mg/kg i.p.), and the left kidney was exposed through a small flank incision. The left renal artery and vein were occluded with a non-traumatic clamp for 45 min. At the end of the ischemic period, the clamp was released for blood reperfusion. SA13353 (3, 10, and 30 mg/kg) and its vehicle (1% methylcellulose) were given orally 30 min before the start of ischemia at a volume 5 ml/kg. In sham-operated control animals, the left kidney was treated identically, except for the clamping. All these surgical procedures were carefully done under the rectal temperature-controlled condition using a heater.

The animals exposed to 45 min of ischemia were housed in metabolic cages 24 h after reperfusion; 5-h urine samples were taken, and blood samples were drawn from the thoracic aorta at the end of the urine collection period. The plasma was separated by centrifugation. These samples were used for measurements of renal function parameters. The kidneys were excised and examined using a light microscope.

In separate experiments, animals were sacrificed at various time points after the start of reperfusion for evaluation of superoxide (O2•−) production, neutrophil infiltration, mRNA expressions, and concentration of IL-10. Renal O2•− production and neutrophil infiltration were evaluated at 6 h after the reperfusion, based on findings reported previously (Nakajima et al., 2006). Renal TNF-α and IL-10 mRNA expression and plasma IL-10 concentration were examined and each 4 h after the reperfusion, based on our recent study (Ueda et al., 2008).

**Renal Functional Parameters.** Blood urea nitrogen (BUN) and plasma creatinine concentration (Pcr) were measured using a commercial assay kit, the BUN-test-Wako and creatinine-test-Wako (Wako Pure Chemicals, Osaka, Japan), respectively. Urine and plasma sodium concentrations were determined using a flame photometer (205D; Hitachi, Hitachinaka, Japan). The fractional excretion of sodium (FE\textsubscript{Na}) was calculated from the following formula: FE\textsubscript{Na} = (UNa \times Pcr/UNa_D × creatinine clearance) × 100, where UNa\textsubscript{D} is the urinary excretion of sodium, and Pcr\textsubscript{D} is the plasma sodium concentration.

**Histological Studies.** Excised left kidneys were processed for light microscopic observation, adhering to standard procedures. The kidneys were fixed in 10% phosphate-buffered 10% formalin, after which the kidneys were sliced into small pieces, embedded in paraffin wax, cut at 4\textmu m, and stained with hematoxylin and eosin. Histological images were analyzed for tubular necrosis and medullary congestion, as described previously (Nakajima et al., 2006). Tubular necrosis was graded as follows: no damage (0), mild damage (1, unicellular, patchy isolated damage), moderate damage (2, damage between 25 and 50%), severe damage (3, damage between 50 and 75%), and very severe damage (4, more than 50% damage). The degree of medullary congestion was defined as: no congestion (0), mild (1, vascular congestion with identification of erythrocytes by \times 400 magnification), moderate (2, vascular congestion with identification of erythrocytes by \times 200 magnification), severe (3, vascular congestion with identification of erythrocytes by \times 100 magnification), and very severe (4, vascular congestion with identification of erythrocytes by \times 40 magnification).

The scoring of the histological data was performed by independent observers in a double-blind manner.

**Neutrophil Infiltration.** Neutrophil infiltration was evaluated using naphthol AS-D chloroacetate esterase staining (91C; Sigma-Aldrich, St. Louis, MO) (Ueda et al., 2008) by counting the number of neutrophils present in the outer zone of the medulla of the kidneys. Neutrophils were counted in 50 randomly selected high-power fields (\times 400) of the outer zone of medulla. Data were expressed as neutrophils per millimeter squared of tissue.

**Measurement of Renal O2•− Production.** Renal O2•− production was measured using a lucigenin-enhanced chemiluminescence assay, as described previously (Nakajima et al., 2006). The whole kidney was removed from rats and cut into strips (2-mm pieces). Immediately afterward, renal tissue segments were placed in test tubes containing modified Krebs-HEPES buffer (pH 7.4, 99.01 mM NaCl, 4.69 mM KCl, 1.18 mM CaCl\textsubscript{2}, 1.30 mM MgSO\textsubscript{4}, 1.03 mM K\textsubscript{2}HPO\textsubscript{4}, 25 mM Na-HEPES, and 11.1 mM glucose) and allowed to equilibrate in the dark for 15 min at 37°C before measurements. After the equilibration, lucigenin (28 \mu M) was added to the tube, and then the luminescence was measured using a luminescence meter (Sirius-2; Berthold Technologies, Bad Wildbad, Germany). The relative light unit was integrated every 3 s for 15 min and averaged. The renal O2•− production was expressed as relative light unit per minute per milligram dry tissue weight.

**Total RNA Extraction, Reverse Transcription, and Real-Time PCR.** Total RNA was isolated from the left kidney of sham-operated control, vehicle-treated ischemic AKI, and drug-treated ischemic AKI groups using commercially available kit (Nippon Gene, Tokyo, Japan) following the manufacturer’s instructions. Total RNA was reverse transcribed using a commercially available kit (Takara Bio, Otsu, Japan). Ten microliters of resulting mixture containing 500 ng of total RNA was incubated at 42°C for 15 min before reverse transcription, then reverse transcription was conducted by incubation at 95°C for 2 min. For real-time PCR, the primer was designed as follows: normal protein S18 forward, 5′-AAGTGGCATCAAGGAGTGATG-3′; Rps18 reverse, 5′-CTCTCTCAGAAGTCATCATGC-3′; TNF-α forward, 5′-ACCTGTGAGAACCGCCTGCTG-3′; TNF-α reverse, 5′-CACCCGTGGTCCTCTGGTAAG-3′; IL-10 forward, 5′-CAGAAAACGACAGGGACTGCTG-3′; IL-10 reverse, 5′-CAAGGGCTGGCAACAGAGGTGAGC-3′; CINC-1 forward, 5′-AGGCTTCGACCTTATCATC-3′; CINC-1 reverse, 5′-AGCCCATGCGTGAATCATC-3′; and Rps18 reverse, 5′-AGCCCATGCGTGAATCATC-3′. All primers were purchased from Takara Bio. The PCR reactions were carried out in an ABI 7000 (Applied Biosystems, Foster City, CA). Reverse transcribed PCR amplifications were performed with a volume of 50 \mu l. Each reaction mixture contained 2 \mu l of cDNA, 25 \mu l SYBR Green PCR master mix (QIAGEN GmbH, Hidden, Germany), and 300 nM each primer. Each reverse transcriptase-PCR run started with a min period at 95°C to activate the Taq polymerase. The annealing was done for 45 cycles. Each cycle consisted of: denaturation at 95°C for 15 s, annealing of primers for 30 s at 55 or 57°C, and elongation for 30 s at 72°C. The mRNA copy numbers were calculated from the cycle threshold value using the ABI Prism 7000 Sequence Detection System (Applied Biosystems). Results of mRNA copy numbers were normalized against Rps18 mRNA.

**Measurements of IL-10 and CINC-1 Protein.** Plasma concentrations of IL-10 and CINC-1 were measured using the Bio-Plex Suspension System (Bio-Rad, Hercules, CA).

**Statistical Analysis.** Values are expressed as the mean ± S.E.M. relevant data were processed by InStat (GraphPad Software Inc., San Diego, CA). For statistical analysis, we used one-way analysis of variance followed by Dunnett’s tests for multiple comparisons. Histological data were analyzed using the Mann-Whitney test. For all comparisons, differences were considered significant at P < 0.05.

**Results**

Screenings for the Target Molecules of SA13353. Although SA13353 was inactive ( < 50% inhibition at 1 \mu M) to most of the tested targets, the compound showed a binding affinity for the rat vanilloid receptor, a candidate target molecule. An IC\textsubscript{50} value of SA13353 for rat vanilloid receptor was 191 ± 28 nM (mean of three independent experiments). Taking into account the K\textsubscript{i} value for the radioligand (0.046 nM), the K\textsubscript{i} value for SA13353 was 35.8 ± 5.2 nM. Capsaicin, the reference for the TRPV1 agonist (Caterina et al., 1997), showed a binding affinity for this receptor, with an IC\textsubscript{50} value of 3140 nM. For further details, please see the online Suppmental Table 1.
Pharmacokinetics and Excretion Route of SA13353. Plasma concentration of SA13353 peaked at 0.5 to 1 h after oral administration and thereafter decreased gradually (Fig. 1A). The mean $C_{\text{max}}$ and $T_{\text{max}}$ values of SA13353 after oral administration at a dose of 30 mg/kg was 476 $+/-$ 135 nM and 0.63 $+/-$ 0.13 h, respectively, and mean area under the plasma concentration-time curve from 0 to 24 h calculated by the linear trapezoidal rule was 3970 $+/-$ 860 nM/h. After oral doses of $[^{14}C]$SA13353 (3 mg/kg) to male rats, 85.09 $+/-$ 1.90 and 8.96 $+/-$ 1.05% of the radioactivity dose were excreted in the feces and urine, respectively, during the 7-day sample collection period, indicating that SA13353 was predominantly excreted into feces (Fig. 1B). The recovery of radioactivity in feces, urine, expired air traps, and cage washings was 95.44 $+/-$ 1.76% of dose during this period. Therefore, we considered that the urinary excretion route is minor, and there is no effect of uninephrectomy on pharmacokinetics of SA13353. In addition, there were not more potent active metabolites than SA13353, and plasma concentrations of metabolites were not higher than that of SA13353 (data not shown). Taking into account the results of the binding and enzyme assays, SA13353 seems to be a near-pure TRPV1 agonist in this study.

Renal Function after the Ischemia/Reperfusion and Effects of SA13353. The renal function of rats subjected to 45-min ischemia showed a marked deterioration when measured 24 h after the reperfusion (Fig. 2). Sham-operated rats, vehicle-treated AKI rats showed significant increase in BUN (173.33 $+/-$ 45.99 mg/dl), Pcr (4.28 $+/-$ 0.41 versus 0.70 $+/-$ 0.15 mg/dl), $FE_{\text{Na}}$ (15.99 $+/-$ 3.84 versus 0.79 $+/-$ 0.18%). The administration of SA13353 (3, 10, and 30 mg/kg) 30 min before ischemia proved significant preventive effects against the development of renal dysfunction. When a higher dose of SA13353 (30 mg/kg) was given, renal function changes induced by ischemia/reperfusion were markedly improved (BUN, 74.59 $+/-$ 0.91 mg/dl; Pcr, 1.92 $+/-$ 0.29 mg/dl; $FE_{\text{Na}}$, 3.40 $+/-$ 0.75%). The administration of SA13353 to sham-operated rats (30 mg/kg) produced no significant changes in the renal function (data not shown).

Histological Renal Damage after the Ischemia/Reperfusion and Effects of SA13353. Histopathological examinations revealed severe lesions in the kidney of vehicle-treated AKI rats (24 h after the ischemia/reperfusion). These changes were characterized by tubular necrosis in the outer zone outer stripe of the medulla (Figs. 3A and 3D; score, 4.00 $+/-$ 0.00) and medullary congestion and hemorrhage in the outer zone inner stripe of the medulla (Figs. 3E and 4B; score, 3.71 $+/-$ 0.18). Pretreatment with SA13353 at 30 mg/kg produced significant preventive effects against the development of tubular necrosis (Figs. 3C and 4A) and medullary congestion and hemorrhage (Figs. 3F and 4B).

Neutrophil Infiltration in the Kidney after the Ischemia/Reperfusion and Effects of SA13353. We evaluated whether pretreatment with SA13353 suppressed the neutrophil infiltration into the renal tissue, an event that has been known to produce $O_2^•$, in and to believe to be one of the main causal factors of the ischemia/reperfusion-induced AKI (Linares et al., 1992). As shown in Fig. 5, neutrophils were observed in the kidneys of vehicle-treated AKI rats 6 h after the reperfusion. The number of infiltration neutrophils in the vehicle-treated AKI rats was significantly increased compared with those in the sham-operated rats (Fig. 5, A and D). To the other hand, the neutrophil infiltration was markedly suppressed by the treatment with SA13353 (30 mg/kg; Fig. 5, C and D).

Renal $O_2^•$ Production after the Ischemia/Reperfusion and Effects of SA13353. We evaluated the effect of preischemic prophylactic treatment with SA13353 on renal $O_2^•$ production. AKI rats were given in Fig. 7, A and C, the increased level of renal CINC-1 mRNA expression at 2 and 4 h after the ischemia/reperfusion was markedly suppressed by the treatment with SA13353. On the other hand, the administration of SA13353 tended to further attenuate the level of IL-10 mRNA expression at 2 and 4 h after the ischemia/reperfusion (Fig. 6, B and E), although these augmentations were not statistically significant. As shown in Fig. 6, A and C, the increased level of renal CINC-1 mRNA expression at 2 and 4 h after the ischemia/reperfusion was partially but significantly (Fig. 7A) suppressed by the treatment with SA13353.

Effects of SA13353 on Plasma IL-10 and CINC-1 mRNA Expression after the Ischemia/Reperfusion. Figure 6, C and F, showed the effect of SA13353 treatment on TNF-$\alpha$, IL-10, and CINC-1 mRNA expression were evaluated at 2 and 4 h after the reperfusion. As shown in Fig. 6, A and D, the increased level of renal TNF-$\alpha$ mRNA expression at 2 and 4 h after the ischemia/reperfusion was markedly suppressed by the treatment with SA13353. On the other hand, the administration of SA13353 tended to further attenuate the increase in IL-10 mRNA expression at 2 and 4 h after the ischemia/reperfusion (Fig. 6, B and E), although these augmentations were not statistically significant. As shown in Fig. 6, A and C, the increased level of renal CINC-1 mRNA expression at 2 and 4 h after the ischemia/reperfusion was partially but significantly (Fig. 7A) suppressed by the treatment with SA13353.

Discussion

Postischemic AKI is a frequent clinical syndrome with a high morbidity and mortality (Kelly and Molitoris, 2000). Reperfusion of previously ischemic renal tissue initiates a series of complex cellular events that results in injury and the eventual death of renal cells because of a combination of...
Grades of score: 0, no change; 1, mild; 2, moderate; 3, severe; and 4, very severe. apoptosis and necrosis (Lieberthal and Levine, 1996). Numerous attempts have been made to prevent AKI using animal models of ischemia/reperfusion-induced renal injury, and various vasodilative agents, including natriuretic peptides, adenosine antagonists, dopamine receptor agonists, calcium antagonists, endothelin receptor antagonists, and acetylcysteine, have been considered to be useful for the prevention and management of AKI (Venkataraman and Koh, 2003), although it remains to be elucidated whether such interventions are beneficial to clinical uses. TRPV1 has also been suggested to play a role in regulating vasodilatation in various vascular beds (Burnett and Lang, 2005). The activation of TRPV1 leads to release of several neuropeptides, such as substance P, dynorphin, and related peptide (CGRP) and somatostatin (Buck and Burks, 1986). CGRP is strongly coexpressed in many TRPV1-expressing nerve fibers, including sensory fibers that innervate the dural vasculature (Dux et al., 2003), and it has been suggested to confer a beneficial counterbalance to the development of hypertension (Márquez-Rodas et al., 2006).

We have already demonstrated that TRPV1 agonists capsaicin and resiniferatoxin potentiate ischemia/reperfusion-induced renal injury in rats (Ueda et al., 2008). Because the oral acute toxicity of capsaicin is relatively high (oral LD₅₀ values of nearly 100–200 mg/kg in mice or rats) (Saito and Yamamoto, 1996), we tried to find a safer TRPV1 agonist than capsaicin, ultimately selecting SA13353 (Murai et al., 2008) from numerous compounds available (oral LD₅₀ values exceed 2000 mg/kg in rats). In the present study, we found that the treatment with SA13353 prevented the ischemia/reperfusion-induced renal dysfunction in rats. We have demonstrated most recently that SA13353 significantly inhibits lipopolysaccharide (LPS)-induced TNF-α production in a dose-dependent manner in rats (Murai et al., 2008). SA13353 inhibited a binding affinity for the rat vanilloid receptor and decreased neuropeptide release from the rat dorsal root ganglion neurons. However, the neuropeptide release by SA13353 was blocked by pretreatment with the TRPV1 antagonist capsazepine. Although SA13353 inhibited LPS-induced TNF-α production in vivo, its effect was not observed in sensory denervation rat and TRPV1 knockout mice. These results suggest that SA13353 inhibits TNF-α production through activation of capsaicin-sensitive afferent neurons mediated via TRPV1 in vivo.

To investigate the possible mechanisms underlying renoprotective effects of SA13353 against the ischemia/reperfusion-induced renal injury, we first evaluated the effects of SA13353 on neutrophil infiltration, which is one of the major causes for renal injury after ischemia/reperfusion. Neutrophil is implicated as a mediator of tissue-destructive events in reperfusion injury (Weiss, 1989). The plasma membrane of the triggered neutrophil is the site of an unusual enzyme, termed the NADPH oxidase, which underlies the cell’s ability to generate a family of reactive oxidation chemicals. The
superoxide radical and its metabolites play an important role in the pathogenesis of tissue injury (Hirota et al., 1990). SA13353 extremely attenuated neutrophil infiltration and O$_2^-$ production, which may be derived mainly from the infiltrating neutrophil after ischemia/reperfusion.

Next, we investigated the possible inhibitory mechanisms of SA13353 on neutrophil infiltration observed in the renal tissue exposed to ischemia/reperfusion. Expressions of adhesion molecules including intercellular adhesion molecule-1 on endothelial cells are important for neutrophil infiltration. For expression of adhesion molecules, some cytokines, including TNF-$\alpha$, have a critical role (Springer, 1990). CINC-1, a major factor in acute inflammation, is responsible for the activation of neutrophils and for neutrophil chemotaxis to sites of injury (Watanabe et al., 1992). On the other hand, the anti-inflammatory cytokine IL-10 has been shown recently to inhibit ischemic and cisplatin-induced AKI (Deng et al., 2001). Therefore, we investigated the effects of SA13353 on TNF-$\alpha$, CINC-1, and IL-10 mRNA expression in the postischemic kidneys. We noted that the preischemic treatment with SA13353 attenuated the ischemia/reperfusion-induced augmentation of TNF-$\alpha$ and CINC-1 mRNA expression level. On the other hand, SA13353 further enhanced the increases in IL-10 mRNA and its serum protein levels elicited by the ischemia/reperfusion. Previous studies have demonstrated that neuropeptides such as CGRP (Feng et al., 1997; Gomes et al., 2005; Harzenetter et al., 2007) and somatostatin (Landa et al., 2005) attenuate renal injury by promoting renoprotection.

Fig. 5. Top, effects of treatment with SA13353 (30 mg/kg) on neutrophil infiltration 6 h after ischemia/reperfusion. Neutrophil infiltration was evaluated using naphthol AS-D chloroacetate esterase staining. Light microscopy of kidneys in the AKI rats treated with vehicle (B), SA13353 (C) 6 h after ischemia/reperfusion and sham-operated rats (A). Arrows, neutrophils (magnification, $\times$400). SA13353 was given orally 30 min before the ischemia. Bottom, effects of treatment with SA13353 (30 mg/kg) on renal superoxide production (E) 6 h after ischemia/reperfusion. SA13353 was given orally 30 min before ischemia. Each column and bar represent the mean $\pm$ S.E.M. *,$P<0.05$; **,$P<0.01$, compared with vehicle-treated AKI rats.

Fig. 6. Effects of treatment with SA13353 (30 mg/kg) on renal TNF-$\alpha$ (A and D), IL-10 mRNA expressions (B and E), and plasma concentration of IL-10 (C and F) 2 (A–C) and 4 (D–F) h after the reperfusion. SA13353 was given orally 30 min before the ischemia. Each column and bar represent the mean $\pm$ S.E.M. *,$P<0.05$; **,$P<0.01$, compared with vehicle-treated AKI rats.
al., 1995) inhibited the LPS-induced TNF-α production in vitro and in vivo. We also found recently that the administration of SA13353 evoked the release of CGRP and somatostatin in rats (Ueda et al., 2008). Taken together, we suggest that the activation of capsaicin-sensitive afferent nerves by SA13353 produces the following release of neuropeptide(s) mainly at the inflammatory reactions after ischemia/reperfusion.

In separate experiments, we confirm that the preventive effects of SA13353 on ischemia/reperfusion-induced renal injury are mediated by TRPV1, as we attempted to utilize the TRPV1 antagonist capsazepine. However, because capsazepine itself affords the ischemia/reperfusion-induced renal injury, we could not evaluate its antagonizing activity on TRPV1 agonist-induced renoprotective effects (unpublished observations). Capsazepine, not only a TRPV1 antagonist, but also inhibits voltage-activated calcium channels and nicotinic acetylcholine receptors (Docherty et al., 1997; Liu and Simon, 1997), thereby suggesting that capsazepine may not be appropriate for antagonizing study in the ischemia/reperfusion-induced renal injury model. Another TRPV1 antagonist, iodo-resiniferatoxin, is also reported to be inadequate for the TRPV1 antagonizing study in vivo (Seabrook et al., 2002). In a preliminary experiment, we noted that iodo-resiniferatoxin inhibited LPS-induced TNF-α production in vivo. Thus, further studies using TRPV1 antagonists with higher pharmacological properties or TRPV1 knockout animals are required to elucidate the TRPV1 mediated renoprotective effect and its precise mechanisms.

In the present study, SA13353 was administered before the start of 45-min ischemia. However, because AKI cannot be predicted in many clinical cases, it is important to evaluate whether TRPV1 agonists can reverse the ischemia/reperfusion-induced renal dysfunction or at least enhance the recovery process when given after the reperfusion. Therefore, we examined the effect of the postischemic treatment (at 30 min after the start of reperfusion) with SA13353 (30 mg/kg p.o.), but the agent produced no significant renoprotective effect (unpublished observations). SA13353 administered after the ischemia/reperfusion may be useful to overcome severe renal injury, although the reasons under further studies using a mild disease model and/or higher doses of SA13353 are required to clarify whether SA13353 treatment after the reperfusion exerts a renoprotective effect.

In conclusion, the preischemic treatment with SA13353 prevents ischemia/reperfusion-induced renal injury, probably via TRPV1-mediated mechanisms, which are closely related to the inhibition of inflammatory responses. Taken together, the previous study using capsaicin and resiniferatoxin (Ueda et al., 2008), renoprotective effects by TRPV1 agonists may be useful to preserve renal function in patients with AKI after cardiac and vascular surgery.

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References


