

MK-571 Attenuates Kidney Ischemia and Reperfusion–Induced Airway Hypersensitivity in Rats

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ABSTRACT

Objective. Reperfusion of the rat kidney has been shown to up-regulate cysteinyl leukotriene-1 receptor, an asthma-associated gene in human bronchioles, and increase expression of leukotriene D₄. In this study, we aimed to investigate the efficacy of MK-571, a leukotriene D₄ inhibitor, against hypersensitivity induced by kidney ischemia and reperfusion (I/R)–associated acute kidney injury.

Methods. Sprague-Dawley male rats were divided into 3 study groups: a sham-operated group, a kidney I/R group, and a group treated with MK-571 before the kidney I/R injury: MK-571 (5 mg/kg) was administered intraperitoneally 15 minutes before ischemia and every 12 hours after reperfusion up to 24 hours. Ischemia was conducted by bilateral occlusion of renal pedicles for 45 minutes, followed by releasing the clamps and closing the abdominal incision. Respiratory function was tested 24 hours after reperfusion, with the use of a 2-chamber whole body plethysmograph for conscious rats. Blood samples, pulmonary bronchoalveolar lavage fluid, and lung tissues were collected at the end of study. In 10 rats, urine was collected at baseline and the end of study.

Results. Compared with the sham group, kidney I/R injury markedly increased enhanced pause (Penh) index during methacholine challenge test ($P < .05$), suggesting airway hypersensitivity; it also increased in inflammatory response and levels of hydroxyl radical production and lipid peroxidation in the lungs. In contrast, in MK-571–treated rats, Penh was muted during methacholine challenge test ($P < .05$).

Conclusions. Kidney I/R injury induces airway hypersensitivity to methacholine challenge test and inflammatory response and oxidative stress in the lungs. Treatment with MK-571, a leukotriene D₄ inhibitor, effectively attenuates airway hypersensitivity, pulmonary inflammatory response, and lung and kidney injury.

MILD asthma has been a frequent symptom associated with acute renal failure (ARF) [1] and acute renal infarction [2]; the mechanism responsible has not been elucidated. Earlier research suggested that renal ischemia and reperfusion (I/R) injury promotes the production of eicosanoids, particularly leukotriene D₄ [3], and up-regulates expression of cysteinyl leukotriene 1 receptor (CysLT1) [4], an important mediator of human bronchial asthma and allergic inflammation, and facilitates activities through 2 distinct G-protein–coupled receptors [5]. We hypothesized that leukotriene D₄ may play an essential role in airway hypersensitivity induced by kidney I/R injury. MK-571 is a leukotriene receptor ligand, and has been

demonstrated capable of inhibiting leukotriene D₄ to activate CysLT1 receptor in human and murine [5].

MK-571, also known as 5-(3-(2-(7-chloroquinolin-2-yl)ethenyl)phenyl)-8-dimethylcarbamyl-4,6-dithiaoctanoic acid

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sodium salt hydrate, has a chemical formula of $C_{26}H_{26}ClN_2NaO_3S_2 \cdot xH_2O$ and a molecular weight of 571.37 Da. The chemical structure of MK-571 is shown in Fig 1A. Previously, MK-571 was shown to attenuate inflammatory

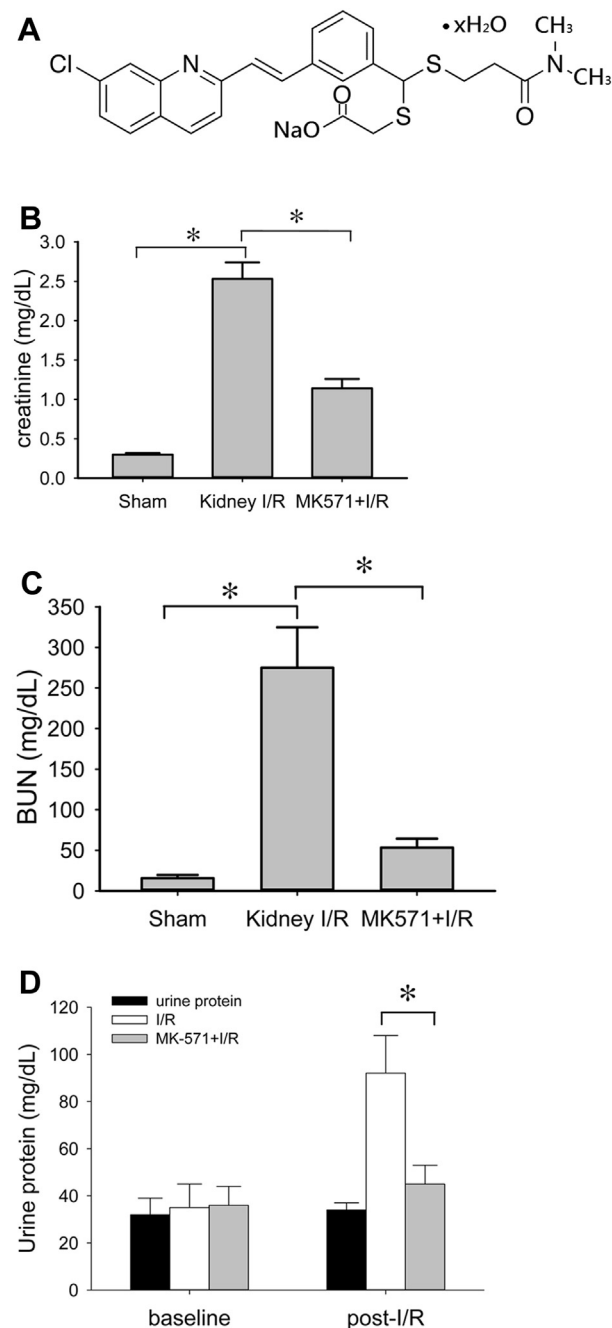


Fig 1. (A) Chemical structure of MK-571 (modified from Sigma-Aldrich data sheet). (B) Bilateral kidney ischemia and reperfusion significantly increases serum creatinine, (C) blood urine nitrogen (BUN) [sham $n = 12$; I/R $n = 14$; MK-571 $n = 14$], and (D) urine protein contents [sham $n = 2$; I/R $n = 4$; MK-571 $n = 4$] compared with the sham group. Treatment with MK-571 markedly reduces kidney injury. * $P < .05$.

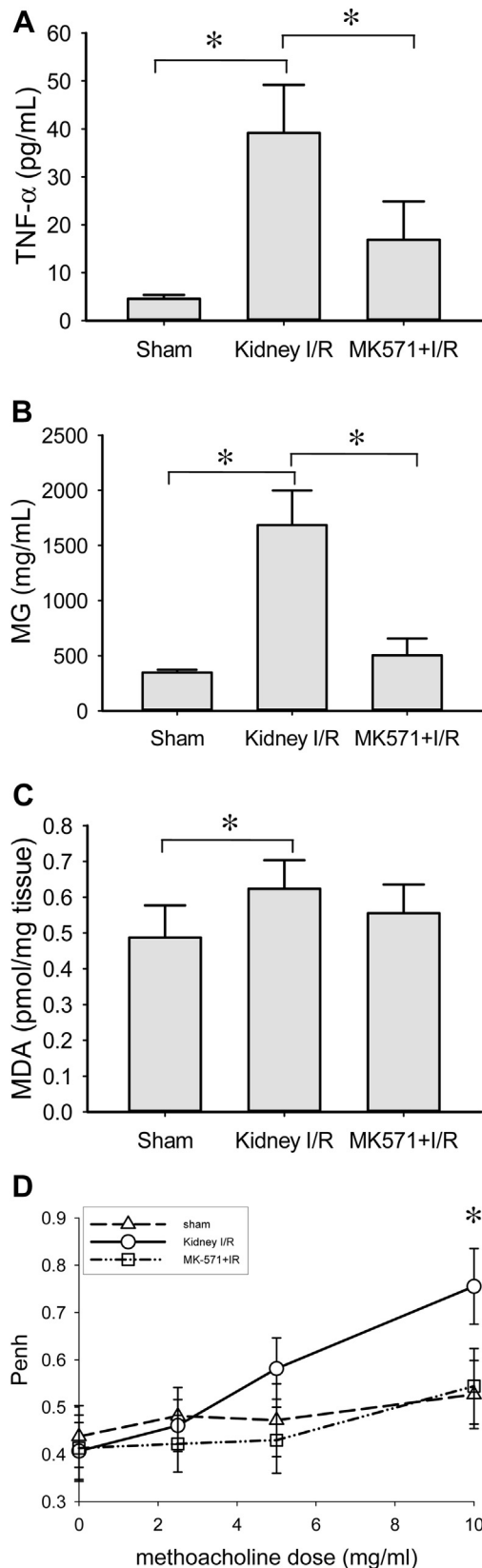
responses through regulating cytokines [5] and adhesion molecules [6] and reducing oxidative stress through preventing export of endothelial oxidized glutathione [7]. In the present study, we aimed to investigate the association between kidney I/R injury and airway hypersensitivity. The former was assessed with the use of serum creatinine and blood urea nitrogen (BUN) level and urine protein contents, and the latter with the use of methacholine challenge test, conducted 24 hours after reperfusion with the use of a 2-chamber whole body plethysmograph for conscious rats. We also assessed the biomarkers for inflammatory response and hydroxyl radical production in the bronchoalveolar lavage fluid (BALF) and oxidative stress in lung tissues, with and without MK-571 treatment.

MATERIALS AND METHODS

Sprague-Dawley rats (250–300 g) were anesthetized with pentobarbital sodium (35 mg/kg intraperitoneally [IP]), followed by a midline laparotomy, through which both renal pedicles were identified and clamped with the use of vascular clips to induce kidney ischemia; the clips were released after 45 minutes of kidney ischemia, and the abdominal incision was then closed with 2 layers of sutures. Rats were released to the cage. Twenty-four hours after reperfusion, respiratory function was assessed: Conscious rats were positioned in a whole body plethysmograph (Buxco Electronics, Sharon, Connecticut), which measures the net of nasal and thoracic flows. Physiologic flows are calculated by taking account of temperature, humidity, and pressure via a proprietary algorithm provided by Buxco, through which the enhanced pause (Penh) index of airway hypersensitivity as an indicator of changes in airway resistance was also calculated. Challenge test was conducted with the use of 4 dosages of methacholine (0, 2.5, 5, and 10 mg/mL). Rats were housed in a separate chamber for 2 minutes, in which methacholine was administered at a certain dose; the rats were then moved to the plethysmograph for Penh assessment. Animals were allowed 10 minutes of rest between challenge tests. After methacholine challenge test, rats were anesthetized in the same procedure as described above. The left femoral artery was cannulated with a polyethylene catheter (PE50), through which blood samples were drawn for serum creatinine and BUN levels. Animals were killed with an overdose of anesthetic (100 mg/kg IP), and the whole lung, including trachea, was carefully removed. A piece of lung tissue at the lower right lobe was dissected and frozen in liquid nitrogen. The right main bronchus was firmly tied and BALF acquired through gently flushing the left lung 3 times with the use of 1.25 mL saline solution through the trachea.

Forty rats were divided into 3 study groups: a sham group ($n = 12$), a renal I/R group ($n = 14$), and a group treated with MK-571 (5 mg/kg IP; $n = 14$) 15 minutes before kidney ischemia and every 12 hours after reperfusion. All animals underwent the same surgical procedure up to bilateral occlusion of renal pedicles.

Pulmonary inflammatory response was assessed with the use of tumor necrosis factor α (TNF- α) and hydroxyl radical production with the use of methylguanidine (MG) in the BALF. The BALF was centrifuged at 10,000g for 10 minutes at 4°C. The levels of MG and TNF- α were examined in the supernate with the use of the spectrofluorimetric method (Jasco 821-FP Fluorescence Detector), with the emission maximum at 500 nm and excitation maximum at 398 nm, and a commercial enzyme-linked immunosorbent assay (ELISA) kit (ab46070; Abcam) following the manufacturer's instructions, respectively.



Pulmonary lipid peroxidation was assessed according to lung tissue malondialdehyde (MDA) level. Lung tissues were homogenized in a lysis buffer, followed by centrifugation at 15,000g for 10 minutes at 4°C. The MDA content was examined in the top supernatant fluid with the use of a commercial ELISA kit (ab118970; Abcam) following the manufacturer's guidelines.

Kidney injury was assessed with the use of serum creatinine and BUN levels and urine protein contents. Blood samples, collected at the end of study, were centrifuged at 10,000 rpm for 10 minutes; the creatinine and BUN levels were examined in the supernate with the use of commercial kits by means of a Fuji Dri-Chem system (4000i; Fujifilm Co). In 10 animals (sham $n = 2$; I/R $n = 4$; MK-571 $n = 4$), urine at baseline before renal pedicle occlusions and at the end of study were collected by a sterilized needle inserted in the bladder to determine urine protein; blood serum and urine were sent to the Union Clinical Laboratory (Taipei, Taiwan) to determine urine protein content and serum creatinine and BUN level.

Data Analysis

Data are presented as mean $\pm$ SEM. Comparisons across more than 2 groups were analyzed with the use of 1-way analysis of variance followed by Dunn test. $P < .05$ was considered to be statistically significant.

RESULTS

In this rat model, we observed apparent kidney injury following bilateral kidney I/R, evident by marked increases in serum creatinine (Fig 1B), BUN (Fig 1C), and urine protein contents (Fig 1D). We also found notable increases in pulmonary inflammatory response, hydroxyl radical production, and lipid peroxidation in the lungs, shown by increases in lavage TNF- α (Fig 2A) and MG level (Fig 2B) and MDA in the lung tissues (Fig 2C), respectively. Fig 2D shows results of methacholine challenge test of the 3 study groups. Penh increases with higher dosage of methacholine; however, the magnitude of increase was much more prominent in the kidney I/R group than in the sham group. In contrast, Penh in MK-571-treated groups was less sensitive to the increase in methacholine dosage, suggesting that treatment with MK-571 attenuates airway hypersensitivity. In addition, our data showed that MK-571 administration markedly reduced lung inflammation and lipid peroxidation in lung tissues, in addition to reduction in kidney I/R injury.

Fig 2. Bilateral kidney ischemia and reperfusion (I/R) increases (A) tumor necrosis factor α (TNF- α) and (B) methylguanidine (MG) levels in the lavage and (C) malondialdehyde (MDA) concentration in the lung tissue compared with the sham group. Treatment with MK-571 reduces degrees of inflammatory response, hydroxyl radical production, and lipid peroxidation in the lungs. (D) Results of methacholine challenge test of 3 groups using 4 dosages (0, 2.5, 5, 10 mg/mL); Penh, enhanced pause, an airway hypersensitivity index, shows marked increase after I/R injury and muted with MK-571 treatment (sham [$n = 12$]; triangles and dashed line; I/R [$n = 14$]; circles and solid line; MK-571 [$n = 14$]; squares and dot-dash-dot line). * $P < .05$.

DISCUSSION

Remote lung injury induced by ischemia and reperfusion of kidneys is well known [8]. However, airway hypersensitivity potentially induced by kidney I/R injury has not been addressed, to our knowledge. In the present rat model, we showed that bilateral kidney I/R can induce airway hypersensitivity, and the mechanism is likely related to the activation of pathways involving the metabolism of arachidonic acid. Matsuyama et al [3] showed that kidney I/R injury up-regulates CysLT1 receptor and induces the cyclooxygenase-3 and 5-lipoxygenase pathway, through which arachidonic acid is metabolized to generate leukotriene A4, which was subsequently converted to leukotriene D4. CysLT1 receptor is known to play an important role in allergic responses in bronchial smooth muscle contraction and asthma airway remodeling, and leukotriene D4 is a ligand specific for the CysLT1 receptor [9]. Furthermore, Nagata et al [9] demonstrated that leukotriene D4 facilitates eosinophil adhesion and transmigration across capillaries via the CysLT1 receptor, thus exacerbating allergic response. In the present study, we also conducted differential cell count in the lung lavage, but did not find conclusive result of increase in lavage eosinophils. In addition to airway sensitivity, we found lung inflammation and increases in hydroxyl radical production and lipid peroxidation in the lungs after kidney I/R injury. Previously, Kelly [10] demonstrated that kidney I/R injury induces immunoreactive interleukin-1 and TNF- α , promoting systemic inflammatory responses and leukocyte activation and infiltration. MK-571 is an effective inhibitor of blocking leukotriene D4 and CysLT1 receptor [5], thus capable of reducing airway hypersensitivity after kidney I/R injury, as demonstrated in the present study. In addition, MK-571 may have antiinflammatory capacity. Failla et al [11] revealed that MK-571 can decrease nuclear factor κ B and TNF- α activation [11], which in turn regulate adhesion molecule expression and cytokines, with which leukocyte recruitment and activation are modulated. As such, blockade of leukotrienes may help to reduce leukocyte infiltration in the lungs, reducing lung inflammation and pulmonary oxidative stress after kidney I/R injury, as observed in our study. On the other hand, Vellenga et al [5] demonstrated that MK-571 is also an effective inhibitor of multidrug resistance protein 1 (MRP1), which belongs to the adenosine triphosphate-binding cassette transporter superfamily, and has been shown to mediate ATP-dependent leukotriene C4

transport and thus reducing leukotriene D4, a metabolite of leukotriene C4 [11]. Recently, van der Deen et al [12] demonstrated that cigarette smoking-induced inflammatory response was markedly attenuated in MRP1-knockout mice, attributed to impairment in leukotriene C4 secretion.

In conclusion, we demonstrated in a rat model that bilateral kidney I/R induce airway hypersensitivity and lung inflammation. Treatment with MK-571 reduced airway hypersensitivity, likely through blocking leukotriene D4 and CysLT1 receptor.

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