Role of Renin-Angiotensin System and Nuclear Factor-κB in the Obstructed Kidney of Rats With Unilateral Ureteral Obstruction

Tatsuya Nakatani1, Satoshi Tamada1,*, Toshihiro Asai1, Yoshihito Iwai1, Taku Kim1, Takashi Tsujino1, Norihiko Kumata1, Junji Uchida1, Koichiro Tashiro1, Nobuyuki Kuwabara1, Toshiyuki Komiy1, Tomohiko Sumi2, Mikio Okamura2 and Katsuyuki Miura3

Departments of 1Urology, 2Nephrology and 3Applied Pharmacology and Therapeutics, Osaka City University, 1-4-3, Asahimachi, Abeno-ku, Osaka 545-8585, Japan

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ABSTRACT—The present study was conducted to elucidate the role of oxidative stress and nuclear factor-κB (NF-κB) in the beneficial effects of angiotensin receptor blockade on obstructive nephropathy. Unilateral ureteral occlusion in rats elicited tubulo-interstitial fibrosis with concurrent macrophage infiltration and increased expression of monocyte chemoattractant protein-1. These changes were accompanied by an induction of renal cortical lipid peroxidation and activation of NF-κB. Both an AT1 antagonist, candesartan, and a NF-κB inhibitor, pyrrolidine dithiocarbamate, markedly attenuated these changes and to a similar extent. These results suggest that the beneficial effects of angiotensin blockade are mediated by the inhibition of oxidative stress and subsequent NF-κB activation in obstructive nephropathy.

Keywords: Unilateral ureteral obstruction, Angiotensin II, Nuclear factor-κB

Interstitial fibrosis is a major histological feature in many progressive renal diseases. The unilateral ureteral obstruction (UUO) animal model is a non-immune, non-proteinuric and non-lipidemic renal disorder that leads to progressive renal tubular atrophy and interstitial fibrosis (1).

Angiotensin-converting enzyme inhibitor (ACEI) and angiotensin receptor blocker (ARB) have been shown to have a beneficial effect in UUO (2). ACEI treatment attenuated interstitial inflammation, suppressed gene expressions of fibrogenic molecules, and ameliorated interstitial fibrosis. While the renin-angiotensin system (RAS) has been implicated in the pathogenesis of interstitial fibrosis in UUO, the precise mechanisms remain unclear.

It is well known that nuclear factor-κB (NF-κB) plays a pivotal role in many inflammatory diseases (3) including renal disease (4, 5). Reactive oxygen species (ROS) has been implicated as a contributing factor in a variety of tissue injuries (6). Furthermore, ROS is a known stimulus of NF-κB activation.

In the present study, we examined the role of RAS and NF-κB in the pathogenesis of obstructive nephropathy. The first aim was to compare the effect of RAS blockade and that of NF-κB inhibition in the pathogenesis of interstitial fibrosis by UUO. The second aim was to investigate the role of ROS and NF-κB in the beneficial effect of RAS blockade. For this purpose, we treated UUO-operated rats with pyrrolidine dithiocarbamate (PDTC) (a putative NF-κB inhibitor with antioxidant property) or ARB (candesartan cilexitil).

The present experiments were conducted in accord with the Guideline Principles for the Care and Use of Laboratory Animals approved by The Japanese Pharmacological Society. Adult male Sprague-Dawley rats weighing 180 to 200 g were used in this experiment. Candesartan cilexitil (candesartan) was a gift from Takeda Chemical Industries, Ltd., Osaka. Candesartan was dissolved in water with CM-cellulose (5 mg/ml) to a final concentration of 3.3 mg/ml. PDTC (Sigma-Aldrich, Tokyo) was dissolved in distilled water to a final concentration of 200 mg/ml. All rats except sham-operated rats underwent unilateral ureteral obstruction. Under sodium pentobarbital (50 mg/kg, i.p.) anesthesia, a midline incision of lower abdomen was made and the left ureter was ligated with 4-0 silk at two points and cut between the ligatures. To examine the effect of ARB and NF-κB inhibition, rats were divided into 4 groups as follows: 1) unilateral ureteral ligated rats (N = 7), 2) unilateral ureteral ligated rats that received candesartan 10 mg/kg per day by gavage 1 h prior to and

*Corresponding author. FAX: +81-6-6646-3048
E-mail: s-tamada@med.osaka-cu.ac.jp
throughout UUO (N = 7), 3) unilateral ureteral ligated rats that received PDTC 200 mg/kg per day by gavage 1 h prior to and throughout UUO (N = 6), 4) sham-operated rats (N = 6). At 5 days after UUO, the left kidney was immediately excised, and the cortices were carefully dissected from the medulla, snap frozen in liquid nitrogen, and stored at −80°C until use for RNA extraction. A portion of renal cortical tissue was processed for measurement of malondialdehyde (MDA) and isolation of nuclear protein, respectively. The remaining renal tissues were fixed in 10% neutral-buffered formalin or methyl Carnoy’s solution. Malondialdehyde in the supernatant of homogenized renal tissues was measured as thiobarbituric acid reactive substances (TBARS) by the colorimetric TBA reaction as described by Ohkawa et al. (7). Nuclear protein extractions and electrophoretic mobility shift assay (EMSA) were performed as described previously (5). The sequence of double-stranded oligonucleotide used for EMSA was as follows: consensus NF-κB, 5′-AGT TGA GGG GAC TTT CCC AGG C-3′ (Promega, Madison, WI, USA). The sections of methyl Carnoy’s solution-fixed renal tissue were stained with mouse monoclonal antibody to the rat monocyte/macrophage (ED-1, 1:500 dilution; Serotec, Oxford, UK) as described previously (5). The sections of formalin-fixed renal tissue sections were incubated with mouse monoclonal antibody to the rat α-smooth muscle actin (α-SMA) (Dako, Carpinteria, CA, USA). Histofine Simple Stain Rat MAX PO (MULTI) (Nichirei Corp., Tokyo) was used as secondary antibody according to manufacturer’s instruction. RNA extracted by the guanidium thiocyanate-phenol-chloroform method, as previously reported (8), was used for Northern blot analysis. A cDNA probe of rat monocyte chemoattractant protein-1 (MCP-1) was used as described previously (9). Data are presented as the mean ± S.E.M. All data were analyzed by using ANOVA and individual comparisons were made by using Duncan’s multiple range test. Statistical significance was defined as P<0.05.

Ureteral ligation caused a prominent injury consisting of tubular dilation, tubular atrophy and severe interstitial fibrosis. Both candesartan and PDTC treatment ameliorated interstitial fibrosis that was estimated by the percentage of α-SMA positive area. The α-SMA protein, which is a marker of tubulointerstitial myofibroblast responsible for

Fig. 1. α-SMA protein expressions in different groups. Photomicrographs show the renal cortex of rats receiving unilateral ureteral obstruction (UUO) (A), UUO with candesartan (B) and UUO with PDTC (C) for 5 days (>200 magnification). D: The percentage of α-SMA protein positive area relative to the whole area was calculated in at least 20 cortical fields (>200 magnification) using a computer-assisted image analysis system (IPAP-WIN; Sumika Technoservice Corp, Hyogo, Japan). Results are expressed as the mean ± S.E.M. *P<0.05 versus sham-operated rats, †P<0.05 versus UUO-operated rats.
a large component of the interstitial collagen deposition after UUO (10), is expressed in the widened interstitial space during UUO (Fig. 1A), whereas it was found only in blood vessels in sham-operated rats (not shown). The percentage of α-SMA positive area in UUO-operated rats was significantly increased compared to that of sham-operated rats (Fig. 1D). Administration of either candesartan or PDTC markedly decreased α-SMA expression (Fig. 1: B, C, D). The inhibitory effect of candesartan was not different from that of PDTC.

Renal cortical NF-κB-DNA binding activity was significantly increased by UUO (Fig. 2). Both candesartan and PDTC attenuated UUO-induced increase in NF-κB-DNA binding. With candesartan treatment, NF-κB-DNA binding activity was decreased to a level not different from that of PDTC-treated rats.

The renal cortical MDA concentration was significantly elevated in UUO-operated rats compared to that of sham-operated rats (0.430 ± 0.047 vs. 0.190 ± 0.016 μMol/mg protein, P<0.05). This increase was completely blunted by either candesartan or PDTC treatment (0.215 ± 0.019, 0.247 ± 0.016, respectively, N.S. compared to that of sham-operated rats).

Monocyte/macrophage infiltration was increased by UUO (Fig. 3A). It was predominantly detected in the widened interstitium around injured tubules. Monocyte/macrophage influx was attenuated by either candesartan or PDTC. In the candesartan-treated group, the number of monocyte/macrophage was not different from that observed in the PDTC-treated group. Gene expression of MCP-1, which is well known as a potent macrophage chemoattractant, was markedly enhanced in the UUO group (Fig. 3B). Both candesartan and PDTC significantly attenuated this increase.

The present results suggest that in the obstructed nephropathy RAS contributes to the development of interstitial inflammation and fibrosis via NF-κB activation.

Activation of NF-κB transcription factor family plays a central role in various chronic inflammatory diseases (3). In our experiment, NF-κB-DNA binding was markedly

Fig. 2. Renal cortical NF-κB binding in different groups by EMSA. Autoradiogram of EMSA is shown in the upper panel. The bracket indicates specific NF-κB-binding complex. The density of each autoradiogram was quantified and shown in the lower panel. Results are expressed as the mean±S.E.M. *P<0.05 versus sham-operated rats, †P<0.05 versus UUO-operated rats. UUO, unilateral ureteral obstruction; ARB, UUO with candesartan; PDTC, UUO with PDTC; Sham, Sham-operated rats.

Fig. 3. Effect of candesartan or PDTC treatment on renal cortical infiltration of monocyte/macrophage. Monocyte/macrophage infiltration was quantified by counting the number of ED-1 positive cells in 20 randomly chosen ×200 magnification areas of renal cortex. Semiquantitative scoring analysis of ED-1 positive cells is shown (A). MCP-1 gene expression is also shown (B). The ordinate shows each mRNA value corrected for the GAPDH mRNA value. Results are expressed as the mean±S.E.M. *P<0.05 versus sham-operated rats, †P<0.05 versus UUO-operated rats. Abbreviations are as given in Fig. 2.
activated by UUO. PDTC treatment in UUO successfully reduced NF-κB-DNA binding activity, and candesartan treatment could also block it. The magnitude of the inhibition was not different between these maneuvers. Previous reports suggested that ACEI treatment or angiotensin II type 1 receptor deficiency inhibited NF-κB activation that was enhanced by UUO (11, 12). The authors speculated that the beneficial effect of RAS blockade might be due to decreased activation of NF-κB, although direct evidence is lacking in their reports. Our present finding confirmed that the beneficial effect of RAS blockade is mediated by the inhibition of NF-κB activation.

ROS is a known source of NF-κB activation (13). As PDTC is one of the antioxidants, these results suggest that enhanced production of ROS following UUO activated NF-κB. The fact that candesartan also blocked MDA concentration and NF-κB strongly suggests that RAS stimulates ROS production that resulted in NF-κB activation. Indeed, a previous report showed that changes in ROS genes expression occur soon after ureteral obstruction (14), suggesting the causative role of activated RAS. Although it is well known that angiotensin II increases NF-κB-dependent up-regulation of MCP-1, a potent macrophage chemoattractant. As NF-κB plays an important role in transcriptional control of MCP-1, NF-κB-dependent up-regulation of MCP-1 expression is one of the likely mechanisms of monocyte/macrophage influx observed in the present study.

In summary, we demonstrated that angiotensin II induced ROS production and sequentially activated NF-κB in the UUO animal model. It is suggested that the beneficial effect of RAS blockade in the pathogenesis of obstructive nephropathy is mediated by the inhibition of oxidative stress and subsequent blockade of NF-κB activation.

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