Antiproteinuric Effect of KD3-671, an Angiotensin II Type 1 Receptor Antagonist, in Rats With Accelerated Passive Heymann Nephritis

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ABSTRACT—The antiproteinuric effect of KD3-671 (2-propyl-8-oxo-1-[(2'-(*H*-tetrazole-5-yl)biphenyl-4-yl)methyl]-4,5,6,7-tetrahydrocycloheptimidazole), an angiotensin II type 1 receptor antagonist, was compared with that of enalapril, an angiotensin II-converting enzyme inhibitor, using an experimental model of membranous nephropathy. KD3-671 (3, 10 and 30 mg/kg per day) and enalapril (30 mg/kg per day) were given p.o. for 40 days, respectively. KD3-671 (30 mg/kg per day) inhibited the elevation of proteinuria and plasma total cholesterol. On the other hand, enalapril showed only a tendency to diminish these parameters. KD3-671 had an antiproteinuric effect in rats with accelerated passive Heymann nephritis. These findings provide considerable encouragement for the clinical development of KD3-671.

Keywords: Angiotensin type 1 receptor antagonist, Antiproteinuric effect, Heymann nephritis

Recently, many investigators reported that angiotensin-converting enzyme (ACE) inhibitors suppressed the progression of diabetic and non-diabetic proteinuric renal disease toward end-stage renal disease (1, 2). Angiotensin II (Ang II) facilitates a variety of physiologic responses that support blood pressure and renal function. On the other hand, excessive generation of Ang II also contributes to the pathogenesis of hypertension, heart failure and renal diseases. There are two pharmacologically distinct subtypes of cell surface receptors for Ang II, designated Ang II type 1 (AT1) receptor and Ang II type 2 (AT2) receptor. AT1 receptors are responsible for the vasoconstriction and the growth promoting effects of Ang II in cultured cells (3). Recent studies report that AT1 receptors are abundant in the glomeruli of rat kidneys (4). On the basis of these findings, we demonstrated that 3 and 10 mg/kg of KD3-671 (2-propyl-8-oxo-1-[(2'-(*H*-tetrazole-5-yl)biphenyl-4-yl)methyl]-4,5,6,7-tetrahydrocycloheptimidazole), an AT1-receptor antagonist, suppressed the progression of experimental anti-glomerular basement membrane antibody associated glomerulonephritis, which resembles rapidly progressive glomerulonephritis in humans (5).

Of the glomerular diseases, idiopathic membranous nephropathy is characterized by diffuse glomerular basement membrane thickening with spike formation without the proliferation of glomerular cells (6). We established

an experimental model of accelerated passive Heymann nephritis, which closely resembles idiopathic membranous nephropathy in humans (7). This experimental model is induced with i.v. injection of rabbit antiserum against Fx1A (an antigen from the brush border of the proximal tubules of rat kidney) following immunization of rats with rabbit gamma globulin in Freund's complete adjuvant in rats. Immediately after the injection of rabbit antiserum against Fx1A antigen, the heterologous phase is caused by binding of rabbit anti-Fx1A antibody to the glomerular epithelial antigen, that is, in situ immune complex formation. Approximately 10 days later, the autologous phase is induced by a reaction between rat antibody against the injected rabbit anti-Fx1A antibody and rabbit anti-Fx1A antibody already bound to the glomeruli.

The aim of the present study was to demonstrate the antiproteinuric effect of KD3-671 in rats with accelerated passive Heymann nephritis and to compare it with that of enalapril, an ACE inhibitor.

Male Sprague-Dawley rats (Nihon SLC, Hamamatsu), weighing 150–170 g, were used in the experiments. These animals were housed in an air-conditioned room at 22 ± 2°C during the experimental period.

KD3-671 was kindly provided by Kotobuki Pharmaceutical Co. (Nagano). The chemical structure of KD3-671 is shown in Fig. 1. Mochizuki et al. reported that KD3-671 displaced specific binding of [¹²⁵I]Sar1 Ile8-AII to AT1 receptor with a K_i value of $0.71 \pm 0.14 \times 10^{-9}$ M in rat liver membranes, but had no affinity for the AT2 receptor

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Fig. 1. Chemical structure of KD3-671.

in bovine cerebellar membranes ($K_i > 10^{-5}$ M) (7). KD3-671 was suspended in 1% methylcellulose (Yoneyama Reagent, Osaka). Enalapril was purchased from Sigma Chemical Co. (St. Louis, MO, USA) and dissolved in distilled water.

Accelerated passive Heymann nephritis was induced as reported previously (8). Briefly, rats were injected with 1.0 ml of rabbit antiserum directed to Fx1A antigen (anti-Fx1A serum) into the tail vein once a day for 2 days starting from the day after the injection of 6.5 mg rabbit gamma-globulin (Sigma Chemical Co.) in 0.25 ml of Freund's complete adjuvant (Difco Laboratories, Detroit, MI, USA) into the hind foot pads. In this experiment, the animals were then divided into five groups ($n=8$). Each of the four groups was given 3, 10 or 30 mg/kg of KD3-671 (APHN + KD3-671) or 30 mg/kg of enalapril (APHN + enalapril). Test drugs were given p.o. daily to rats from the day of anti-Fx1A serum injection (the 1st day) to the 40th day. The accelerated passive Heymann nephritic group (APHN) was given orally distilled water. In addition, a control group ($n=8$) that did not receive the anti-Fx1A serum was used for comparison with the nephritic group.

The 24-h urine samples were obtained by keeping each animal in an individual metabolic cage for 24 h without food and water. The urine was then centrifuged and the supernatant was used for the determination of protein. The urinary protein content was determined by the method of Kingsbury et al. (9) and expressed as mg/24-h urine. Immediately after the last collection of urine samples, blood samples were taken from the renal vein under pentobarbital anesthesia with a heparinized microsyringe. The blood was centrifuged at $2,250 \times g$ for 20 min at 4°C to obtain the plasma for the determination of urea nitrogen and total cholesterol. The plasma urea nitrogen content was determined with a commercial assay kit (BUN Kainos®; Kainos, Tokyo) and is expressed as mg/dl plasma. The plasma cholesterol content was determined with the method of Zurkowski (10) and is expressed as mg/dl plasma.

For light microscopic study, both kidneys were taken

on the 40th day. The kidneys were fixed in alcohol and the tissues embedded in paraffin were then cut into $2-3\text{-}\mu\text{m}$ -thick sections. The sections were stained with periodic acid-methenamine silver (PAM) and anti-Thy 1 antibody, respectively. For evaluating the thickening of glomerular basement membrane (thickening), the PAM-positive area in glomeruli was measured in twenty-five glomeruli per section using an image-analyzer (TOYOCO Imageanalyzer VI®; Toyobo, Tokyo), and the Thy 1-positive area in glomeruli was also measured and then subtracted from the PAM-positive area. The thickening is expressed as $\text{mm}^2/\text{glomerular cross section (GCS)}$.

The results obtained are expressed as the mean $\pm$ S.D. The data were analyzed by one-way analysis of variance (ANOVA) or the Kruskal-Wallis test. To determine the significance of differences among the groups, the Bonferroni multiple comparison test was used. Differences with $P < 0.05$ were considered to be significant.

We detected no abnormalities in general body appearance and body weight of each nephritic animal treated with test drugs. As shown in Fig. 2, when test drugs were given from the day after the anti-Fx1A serum (the 1st day), KD3-671 (30 mg/kg per day) significantly suppressed the urinary protein excretion by 67% on the 40th day. As shown in Table 1, KD3-671 (30 mg/kg per day) significantly inhibited the elevation of plasma total cholesterol, but not urea nitrogen on the 40th day. Enalapril (30 mg/kg per day) showed a tendency to diminish the urinary protein excretion. Enalapril (35 mg/kg per day) was reported to decrease proteinuria in the passive Heymann nephritic

Fig. 2. Effect of KD3-671 on urinary protein excretion of rats with accelerated passive Heymann nephritis. KD3-671 or enalapril was given from day 1 after i.v. injection of anti-Fx1A serum. Columns: (a) control, (b) APHN, (c) APHN + KD3-671 (3 mg/kg), (d) APHN + KD3-671 (10 mg/kg), (e) APHN + KD3-671 (30 mg/kg) and (f) APHN + enalapril (30 mg/kg). Each plot denotes the means $\pm$ S.D. of 8 rats. * $P < 0.05$ versus APHN (b).

Table 1. Effects of KD3-671 on plasma urea nitrogen, plasma cholesterol and thickening of glomerular basement membrane in rats with accelerated passive Heymann nephritis

Treatment (mg/kg per day, p.o.)		n	Urea nitrogen (mg/dl)	Cholesterol (mg/dl)	Thickening of glomerular basement membrane ($\times 10^{-3}$ mm ² /GCS)
Control	—	8	18.4 $\pm$ 1.5	69.4 $\pm$ 15.0	0.64 $\pm$ 0.06
APHN	—	8	19.0 $\pm$ 1.3	150.8 $\pm$ 47.2 [#]	1.20 $\pm$ 0.15 ^{##}
APHN + KD3-671	3	8	19.1 $\pm$ 1.3	146.3 $\pm$ 31.5	1.27 $\pm$ 0.22
APHN + KD3-671	10	8	20.1 $\pm$ 1.8	132.5 $\pm$ 43.6	1.22 $\pm$ 0.17
APHN + KD3-671	30	8	20.1 $\pm$ 1.7	99.4 $\pm$ 20.3*	1.14 $\pm$ 0.12
APHN + enalapril	30	8	19.6 $\pm$ 1.8	108.1 $\pm$ 34.9	1.34 $\pm$ 0.27

Test drugs were given p.o. daily to rats from the day after injection of anti-Fx1A serum to the 40th day. Plasma and kidney were taken on the 40th day for biochemical and light microscopic studies, respectively. Each value is a mean $\pm$ S.D. and n indicates the number of rats used. APHN and GCS indicate the accelerated passive Heymann nephritic rat and glomerular cross-section, respectively. [#] and ^{##} indicate significant differences from the control at $P < 0.05$ and $P < 0.01$, respectively. * indicates a significant difference from the APHN at $P < 0.05$.

model (11). This difference may result from the dose of enalapril used and/or severity of glomerular alterations. Amano et al. demonstrated that repeated administration of KD3-671 (10 mg/kg) to 15-week-old stroke-prone spontaneously hypertensive rats for 7 weeks did not affect renal function during the experimental period (12). In this study, KD3-671 (30 mg/kg per day) had no effect on urea nitrogen, which was measured for evaluating renal function. Therefore, it is possible that the antiproteinuric effect of KD3-671 may not have necessarily resulted from reduction of renal function. Long-term administration of KD3-671 slightly reduced the thickening of glomerular basement membrane (Table 1). Our previous study indicated that KD3-671 also suppressed the excessive expression of proteoglycan and fibronectin in glomeruli in the rat anti-glomerular basement membrane nephritic model (5). AT1 receptor antagonist reduces TGF- β and collagen IV mRNA in glomeruli in diabetic rat model (13). Collagen IV is one of crucial components of the glomerular basement membrane. Thus, while we did not measure the glomerular TGF- β synthesis in this study, the effect of KD3-671 on the development of proteinuria may be partly due to decreasing glomerular TGF- β synthesis in addition to its hypotensive effect on intraglomerular pressure.

In summary, we demonstrated that KD3-671 had an antiproteinuric effect in rat accelerated passive Heymann nephritis. It has been well recognized that proteinuria is the best independent predictor of both disease progression and end-stage renal disease in chronic nephropathies (14). These findings would provide considerable encouragement for the possible application of KD3-671.

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