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Angiotensin II type I receptor antagonist suppresses proteinuria and glomerular lesions in experimental nephritis

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Abstract

Angiotensin-converting enzyme inhibitors exert a beneficial effect on nephritis. We investigated the effects of KD3-671, an angiotensin AT_1 receptor antagonist (2-propyl-8-oxo-1-[(2′-(1*H*-tetrazole-5-yl)biphenyl-4-yl)methyl]-4,5,6,7-tetrahydro-cycloheptimidazole), on anti-glomerular basement membrane antibody-associated nephritis in rats. Untreated nephritic rats had massive proteinuria, glomerular lesions including crescent formation, a significant augmentation of proliferating cell nuclear antigen-positive cells, α-smooth muscle actin-positive cells, and the increase in deposition of proteoglycan, fibronectin and desmin in the glomeruli. Administration of KD3-671 to nephritic rats prevented the development of intense proteinuria, glomerular alterations and the increase in plasma urea nitrogen. KD3-671 suppressed the deposition of matrix protein and the expression of α-smooth muscle actin and desmin in the nephritic glomeruli. Captopril, an angiotensin-converting enzyme inhibitor, suppressed urinary protein excretion and the expression of desmin in the nephritic glomeruli, but not other parameters. These results suggest that KD3-671 may be a useful medicine against glomerulonephritis and glomerulosclerosis.

Keywords: Anti-glomerular basement membrane antibody-associated nephritis; Angiotensin AT_1 receptor antagonist; Proliferation; Extracellular matrix protein; Transformed cell

1. Introduction

The renin–angiotensin system regulates systemic and glomerular capillary pressure (Neuringer and Brenner, 1993), and glomerular permeability (Keane and Raij, 1985). Many investigators have reported a beneficial effect of an angiotensin-converting enzyme inhibitor on glomerulonephritis (Savage and Schreier, 1991; Remuzzi et al., 1993), diabetic nephropathy (Morelli et al., 1990; Remuzzi et al., 1991), and remnant kidney model (Brunner, 1992), suggesting that angiotensin II plays one of the pivotal roles in the development of renal dysfunction in kidney diseases.

Angiotensin II stimulates mesangial cells to generate growth factors, including transforming growth factor (TGF)-β which participates in tissue remodeling and sclerosis (Nahas, 1992; Kagami et al., 1994). Furthermore, angiotensin II promotes mesangial cell proliferation (Ray et al., 1991; Wolf and Neilson, 1993), hypertrophy (Anderson et al., 1993), and production of extracellular matrix proteins in transformed cells (Yamamoto et al., 1994). Ray et al. (1994) reported that angiotensin II directly stimulates human fetal mesangial cells through angiotensin II receptors.

There are two pharmacologically distinct subtypes of cell surface receptors for angiotensin II, designated angiotensin AT_1 receptor and angiotensin AT_2 receptor. Angiotensin II evokes most of the major biological responses in vivo and in vitro through angiotensin AT_1 receptors. The angiotensin AT_1 receptors are responsible for the vasoconstriction and the growth-promoting effects of angiotensin II in cultured cells (Bernstein and Berk, 1993). There are recent reports that angiotensin AT_1 receptors are abundant in the glomeruli of rat kidneys (Sechi et al., 1992; Meister et al., 1993). Several non-peptidic angiotensin II receptor antagonists have been developed. One of them, SC-51316 (an angiotensin AT_1 receptor antagonist, 2,5-dibutyl-2,4-dihydro-4-[[2-(1*H*-tetrazol-5-yl)[1,1′-biphenyl-4′-yl]methyl]-3*H*-1,2,4-triazol-3-one) ameliorates renal tubulointerstitial fibrosis caused by unilateral ureteral obstruction in rats (Ishidoya et al., 1995). Treatment of rats with losartan, an angiotensin AT_1 receptor antagonist, starting at the time of reperfusion after renal ischemia,

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caused a significant decrease in serum creatinine, suggesting that losartan accelerates recovery of renal function (Kontogiannis and Burns, 1998). L-158,809 (an angiotensin AT_1 receptor antagonist, 5,7-dimethyl-2-ethyl-3(−)[[2′-(1*H*-tetrazol-5-yl)biphenyl-4-yl]methyl]-imidazo-(4,5-*b*)pyridine) also prevents the increase of proliferating cell nuclear antigen-positive and ED-1-positive cells in the glomeruli in experimental nephritis induced by the injection of anti-thymocyte serum (Zoja et al., 1998). Therefore, angiotensin AT_1 receptors are likely involved in the development of renal injury and an angiotensin AT_1 receptor antagonist seems to be a promising antinephritic agent.

Anti-glomerular basement membrane antibody-associated nephritis is characterized by biphasic heavy proteinuria, severe glomerular alterations, intense cellular proliferation and mesangial matrix expansion, and leukocytes infiltrating into the glomeruli (Hayashi et al., 1994a,b). It is reported that the plasma angiotensinogen levels rose rapidly after the i.v. injection of anti-glomerular basement membrane serum (Yayama et al., 1995a), and TGF-β mRNA levels rose in the renal cortex of anti-glomerular basement membrane antibody-associated nephritic rats (Yayama et al., 1995b). In the present studies, we investigated whether KD3-671, an angiotensin AT_1 receptor antagonist, which is a cycloheptimidazol derivative (2-propyl-8-oxo-1-[(2′-(1*H*-tetrazole-5-yl)biphenyl-4-yl)methyl]-4,5,6,7-tetrahydro-cycloheptimidazole, Mochizuki et al., 1995), suppresses proteinuria and glomerular injury. Moreover, to elucidate the effects of KD3-671 on cellular proliferation and matrix expansion, we determined the number of proliferating cell nuclear antigen-positive cells, deposition of proteoglycan and fibronectin, and expression of α-smooth muscle actin and desmin in the nephritic glomeruli treated with KD3-671.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley strain rats, weighing 150–170 g (Nihon SLC, Hamamatsu, Japan), were used for all experiments. These animals were housed in an air-conditioned room at 23 ± 1°C during the experimental period.

2.2. Drugs

KD3-671 was kindly provided by Kotobuki Pharmaceutical (Nagano, Japan). The chemical structure of KD3-671, 2-propyl-1-[(2′-(1*H*-tetrazole-5-yl)biphenyl-4-yl) methyl]-4,5,6,7-tetrahydro-cycloheptimidazole is shown in Fig. 1. KD3-671 was suspended in 1% methylcellulose (Yoneyama Regent, Osaka, Japan). Captopril was purchased from Sigma (St. Louis, MO, USA) and was dissolved in distilled water.

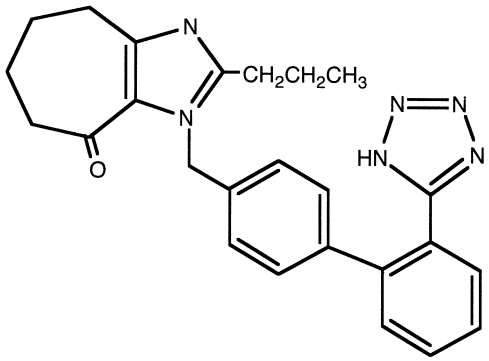

Fig. 1. Chemical structure of KD3-671, 2-propyl-8-oxo-1-[(2′-(1*H*-tetrazole-5-yl)biphenyl-4-yl)methyl]-4,5,6,7,8-tetrahydro-cycoheptimidazole. MW: 426.5.

2.3. Experimental protocol (Nagao et al., 1994)

2.3.1. Induction of anti-glomerular basement membrane antibody-associated nephritis and treatment with test drug

Anti-glomerular basement membrane antibody-associated nephritis was induced in rats by injection of 6.5 mg rabbit γ-globulin (Sigma) in 0.25 ml of Freund's complete adjuvant into the hind foot pads, following the injection of 0.6 ml of rabbit anti-rat glomerular basement membrane serum into the tail vein, in accordance with the method reported previously (Hayashi et al., 1994a). Urine samples were collected, and the rats were divided into five groups of eight rats such that the average protein content in the 24-h urine of each group was similar. Four groups were given orally 1, 3 or 10 mg kg^{-1} day^{-1} of KD3-671 or 100 mg kg^{-1} day^{-1} of captopril, respectively in a volume of 1 ml per 100 g body weight, daily from day 1 after the i.v. injection of anti-glomerular basement membrane serum to day 40. The remaining group was given orally the vehicle (distilled water) instead of test drugs and served as the nephritic control. In addition, a non-treated (normal) group was used for comparison with the nephritic group.

2.3.2. Urine and blood collection

The urine samples were obtained by keeping each animal in an individual metabolic cage for 24 h without food and water on days 1, 5, 10, 20, 30 and 40 as previously reported (Hayashi et al., 1994a). At the beginning of the urine collection, the rats were given orally 8 ml of distilled water. Blood samples were obtained on day 41 after the urine collection. Blood was drawn from the abdominal aorta under pentobarbital (40 mg/kg, i.p., Tokyo Kasei, Tokyo, Japan) anesthesia, and then centrifuged at 2250 × *g* to obtain plasma.

2.4. Determinations of urinary protein, and plasma urea nitrogen and antibody levels against rabbit immunoglobulin G

Urinary protein was determined by the method of Kingsbury et al. (1926) and expressed as milligrams per day. Plasma urea nitrogen was determined with a commer-

cial assay kit (BUN Kainos, Kainos, Tokyo, Japan) and expressed as milligrams per deciliter. The measurement of plasma antibody levels against rabbit immunoglobulin G was performed by enzyme-linked immunosorbent assay (Hayashi et al., 1994b).

2.5. Histopathological examination

For light microscopic study, the kidneys isolated from rats anesthetized with pentobarbital on day 41 were fixed in 10% formalin in 0.01 M phosphate-buffered saline (PBS) pH 7.4 and dehydrated by immersing them stepwise into various concentrations of ethyl alcohol from low to high. The tissues were then embedded in paraffin and sectioned into 2- to 3-μm thick slices, and the sections were stained with Masson's trichrome or hematoxylin-eosine. The evaluation was performed by a person who did not know the identity of sections. The number of nuclei (hypercellularity) and crescent formation, adhesion of Bowman's capsule to capillary wall (adhesion) and fibrinoid necrosis in the glomeruli were observed under a light microscope. To assess these parameters, an equatorial cross-section was selected by random sampling. The number of nuclei was counted and expressed as the mean number per glomerular cross-section in 30 glomeruli/section. Fifty glomeruli/section were observed under a light microscope to evaluate crescent formation, adhesion and fibrinoid necrosis, respectively. The respective histological parameters and the index of glomerular injury were calculated as reported earlier (Hayashi et al., 1994b; Nagao et al., 1994). Briefly, the extent of crescent formation, adhesion and fibrinoid necrosis was scored as 1 (mild), 2 (moderate) or 3 (severe). The number of glomeruli corresponding to each score is given as n_1, n_2 and n_3. A crescent formation index (CI), an adhesion index (AI) and a fibrinoid necrosis index (FI) were calculated from the following formula.

$$\text{CI, AI and FI} = 1n_1 + 2n_2 + 3n_3.$$

The index of glomerular injury was calculated for evaluating the extent of glomerular alterations as follows:

$$\text{Index of glomerular injury} = \frac{(3 \times \text{CI}) + (2 \times \text{AI}) + (1 \times \text{FI})}{(3 + 2 + 1) \times 50}$$

We gave 3, 2 and 1 points to CI, AI and FI, respectively, because we consider that CI, AI and FI are associated with severity of glomerular lesions in this order; and '50' is the number of glomeruli.

2.6. Immunohistochemical examination

Paraffin sections for immunoenzymatic staining of rat-immunoglobulin G, proliferating cell nuclear antigen, proteoglycan, fibronectin, α-smooth muscle actin and desmin, were treated with 0.1% protease (Sigma) in 0.05 M Tris–HCl buffer for 7 min, washed in chilled 0.01 M PBS pH 7.4. The sections were then incubated with the following monoclonal antibodies: mouse anti-rat immunoglobulin G monoclonal antibody (Cappel, West Chester, PA, USA), mouse anti-proliferating cell nuclear antigen monoclonal antibody (Coulter Immunology, Hialeal, FL, USA), mouse anti-proteoglycan monoclonal antibody (Seikagaku Kogyo, Tokyo, Japan), goat anti-fibronectin monoclonal antibody (Calbiochem, La Jolla, CA, USA), mouse anti-smooth muscle actin monoclonal antibody (Dako, Glostrup, Denmark), and mouse anti-desmin monoclonal antibody (Dako), at a dilution of 1:100 for 60 min. The sections were washed again with 0.01 M PBS, treated with 0.3% hydrogen peroxide in methanol for 20 min to block endogenous peroxidase, and incubated with biotinylated affinity purified anti-mouse or goat immunoglobulin G and avidinated horseradish peroxidase with 3,3′-diaminobenzidine tetrahydrochloride (Vectastain ABC Kit, Vector Institution, Burlingam, CA, USA). All steps were carried out at room temperature.

The total area of immunoreactive rat-immunoglobulin G, desmin, proteoglycan or fibronectin in the glomeruli was measured in 30 glomeruli per section using an image analyzer (Toyobo Image Analyzer V1, Toyobo, Tokyo, Japan) and presented as $\text{mm}^2/1 \times 10^{-2}$ mm^2. Proliferating cell nuclear antigen-positive cells and α-smooth muscle actin-positive cells in the glomeruli were counted with an image analyzer (30 glomeruli/section), and the results were expressed as the number of cells/100 cells.

2.7. Statistical analysis

The data represent the means ± S.D., and the results were statistically evaluated using Stat View 4.5 (Abacus

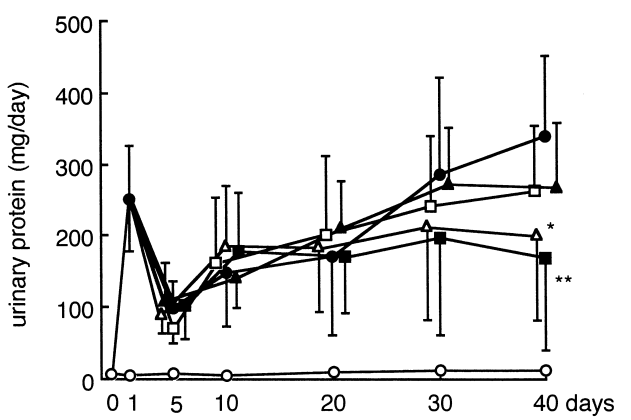

Fig. 2. Effect of KD3-671 on urinary protein excretion of rats with anti-glomerular basement membrane antibody-associated glomerulonephritis. Test drugs were given p.o. daily during the period from day 1 after i.v. injection of anti-glomerular basement membrane serum to day 40. (○): normal, (●): nephritic control, (▲): nephritis + KD3-671 (1 mg kg^{-1} day^{-1}, p.o.), (□): nephritis + KD3-671 (3 mg kg^{-1} day^{-1}, p.o.), (■): nephritis + KD3-671 (10 mg kg^{-1} day^{-1}, p.o.), (△): nephritis + captopril (100 mg kg^{-1} day^{-1}, p.o.). Each plot shows the means ± S.D. for eight rats. $^{*}P < 0.05$ and $^{**}P < 0.01$, compared to the nephritic control group.

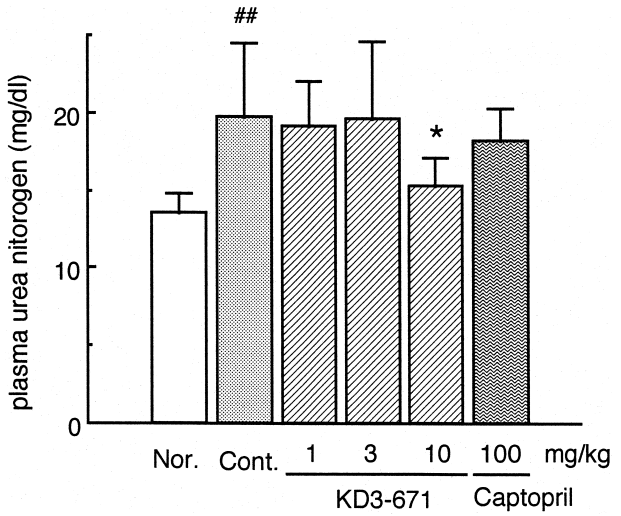

Fig. 3. Effect of KD3-671 on plasma urea nitrogen content of rats with anti-glomerular basement membrane antibody-associated glomerulonephritis. Test drugs were given p.o. daily during the period from day 1 after i.v. injection of anti-glomerular basement membrane serum to day 40. Each column plot gives the means ± S.D. for eight rats. $\#\#P < 0.01$, compared to the normal group. $*P < 0.05$ and $**P < 0.01$, compared to the nephritic control group.

Concept, Berkeley, CA, USA). The data were analyzed by one-way analysis of variance (ANOVA). To determine the significance of differences between the groups, the Bonferroni multiple comparison test was used. Differences with $P < 0.05$ were considered to be significant.

3. Results

3.1. Urinary protein excretion and plasma urea nitrogen (Figs. 2 and 3)

When the treatment with KD3-671 was started from the day after the injection of anti-glomerular basement membrane serum, KD3-671 at 10 mg/kg had significantly suppressed urinary protein excretion by 52% on day 40. The administration of captopril at 100 mg/kg also significantly suppressed proteinuria by 43% on day 40. In addition, on day 40, KD3-671 at 10 mg/kg had inhibited the increase of plasma urea nitrogen content by 72% but captopril had had no effect.

3.2. Histological examination (Figs. 4–6)

Light microscopic examination of the nephritic glomeruli revealed lesions characterized by severe crescent formation, adhesion, fibrinoid necrosis and proliferation of mesangial cells. KD3-671 at 10 mg/kg reduced the incidence of crescent formation and adhesion, and the index of glomerular lesion in the glomeruli on day 40. However, captopril did not affect glomerular alterations.

Glomerular cell proliferation, namely hypercellularity and an increase of proliferating cell nuclear antigen-positive cells in the glomeruli, was observed in the nephritic rats (hypercellularity: the nephritic control 59.7 ± 4.3

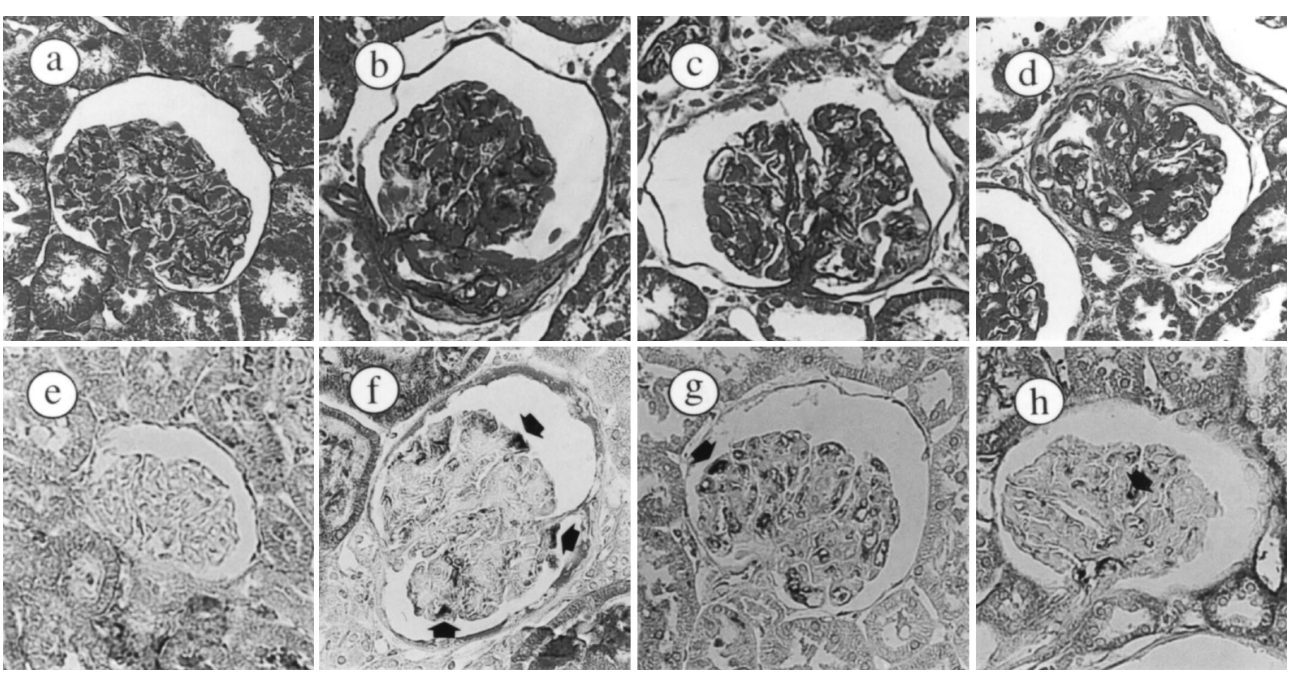

Fig. 4. Light micrographs of glomeruli from rats of the normal group (a, e), the nephritic control group (b, f), the group given KD3-671 (10 mg kg^{-1} day^{-1}, p.o.) (c, g), and the group given captopril (100 mg/kg, p.o.) (d, h). KD3-671 or captopril was given from day 1 after i.v. injection of anti-glomerular basement membrane serum. The rats were examined on day 40 after i.v. injection of anti-glomerular basement membrane serum (Masson's trichrome stain, ×400; a, b, c, d), (immunohistochemical glomerular staining for proliferating cell nuclear antigen, arrow, ×400; e, f, g, h). Note that crescent formation and the increase in the number of proliferating cell nuclear antigen-positive cells are markedly less in the group treated with KD3-671 than in the nephritic control group.

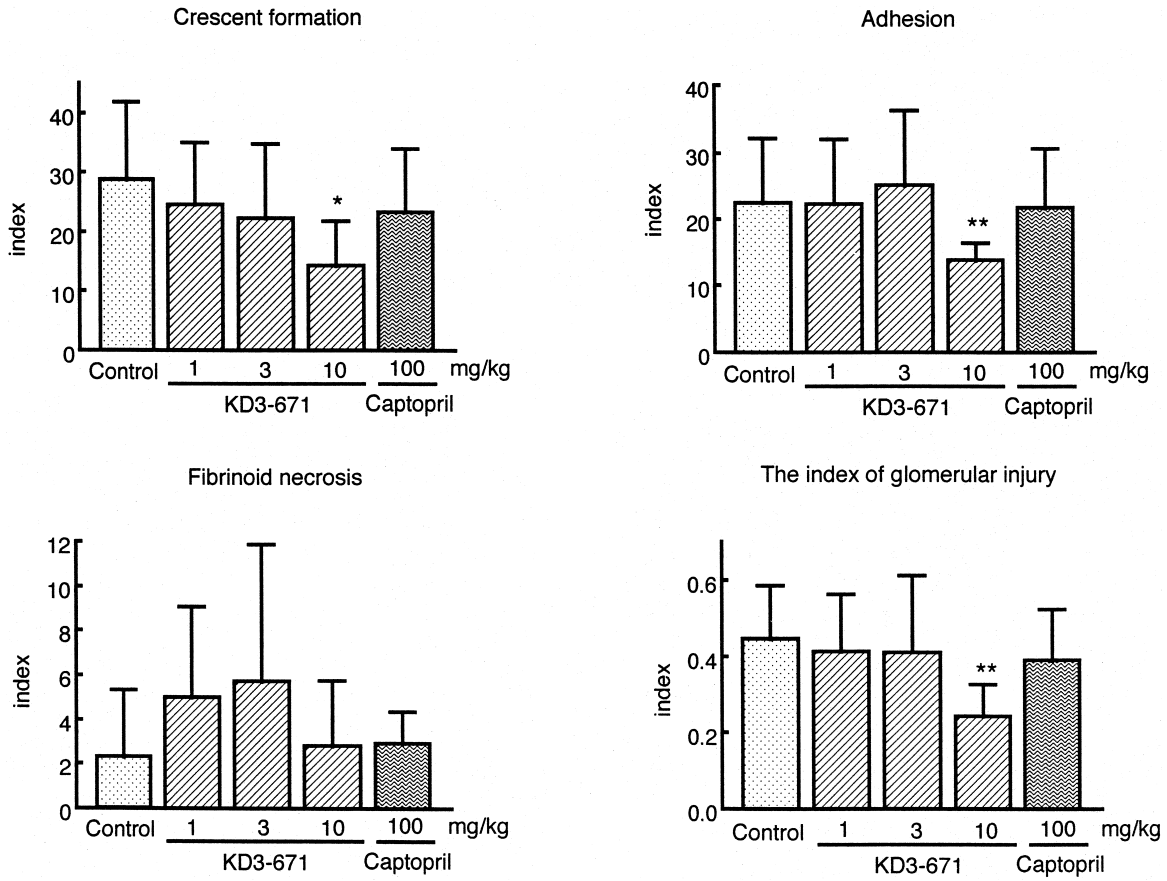

Fig. 5. Effect of KD3-671 on histopathological parameters of rats with anti-glomerular basement membrane antibody-associated glomerulonephritis. Test drugs were given p.o. daily during the period from day 1 after i.v. injection of anti-glomerular basement membrane serum to day 40. Each column plot gives the means ± S.D. for eight rats. $^{*}P < 0.05$ and $^{**}P < 0.01$, compared to the nephritic control group.

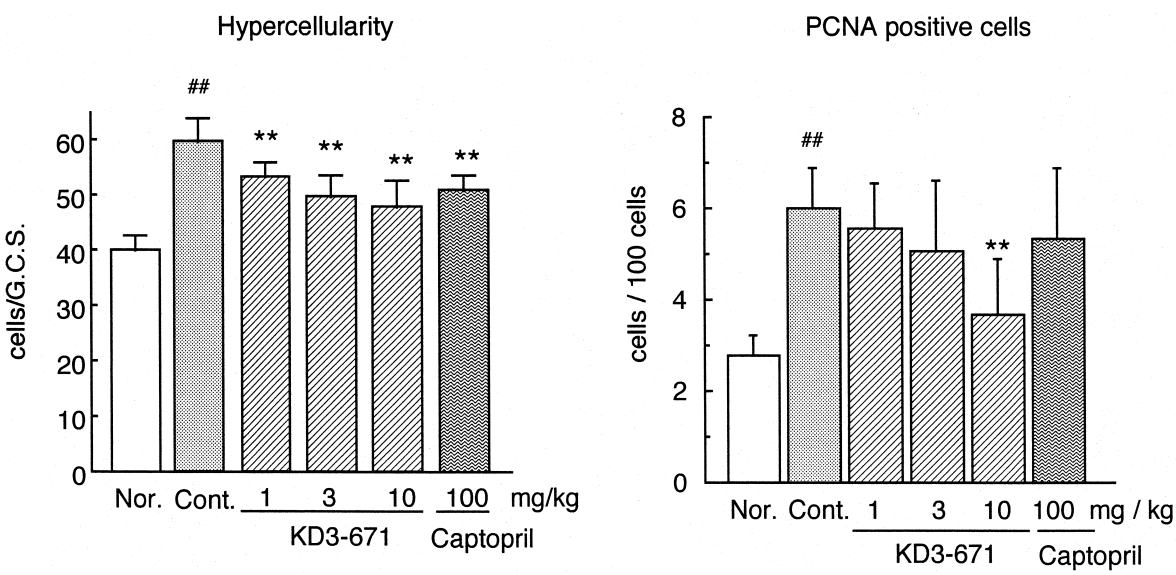

Fig. 6. Effect of KD3-671 on hypercellularity and the increase in proliferating (proliferating cell nuclear antigen-positive) cells of rats with anti-glomerular basement membrane antibody-associated glomerulonephritis. Test drugs were given p.o. daily during the period from day 1 after i.v. injection of anti-glomerular basement membrane serum to day 40. Each column plot gives the means ± S.D. for eight rats. The results indicate the number of cells/glomerular cross-section (G.C.S.) in 30 glomeruli/section for hypercellularity and the number of cells/100 cells for proliferating cell nuclear antigen-positive cells. $\#\#P < 0.01$, compared to the normal group. $^{*}P < 0.05$ and $^{**}P < 0.01$, compared to the nephritic control group.

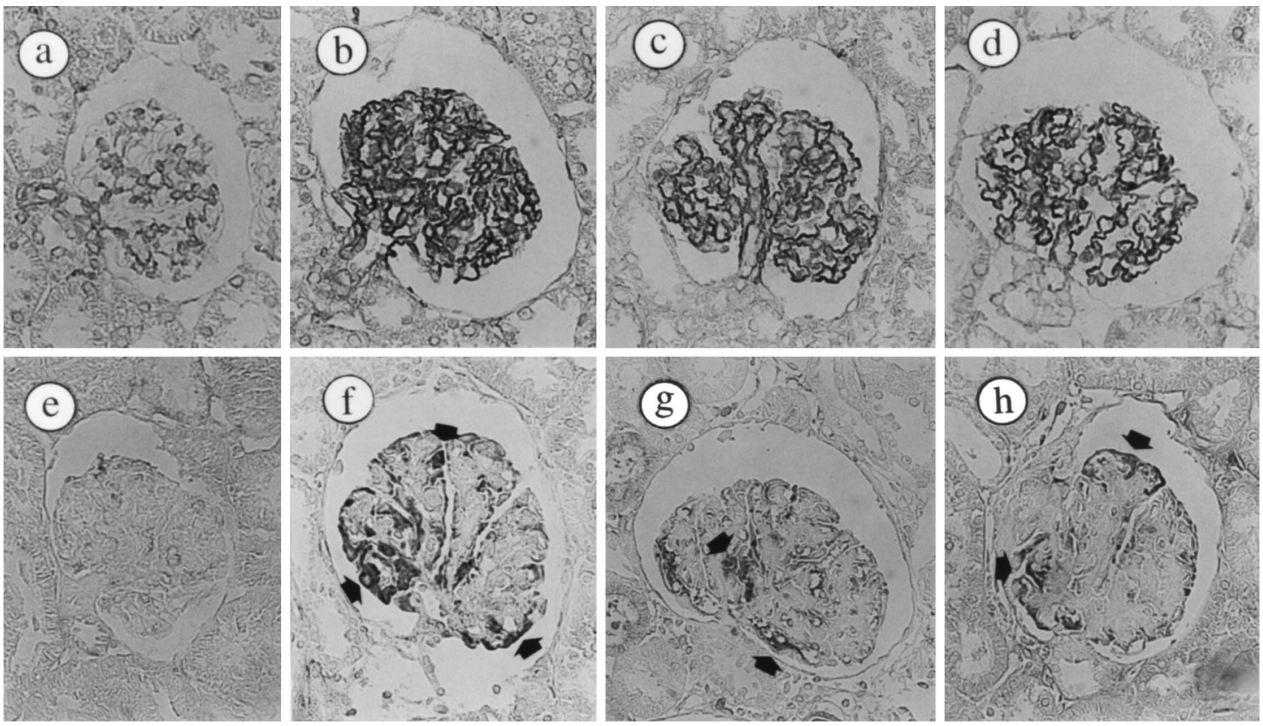

Fig. 7. Photographs of glomeruli immunohistochemically stained with anti-proteoglycan and anti-desmin monoclonal antibodies. The glomeruli were obtained on day 40 after i.v. injection of anti-glomerular basement membrane serum. (a, e) normal, (b, f) nephritic control, (c, g) nephritis + KD3-671 (30 mg kg^{-1} day^{-1}, p.o.), (d, h) nephritis + captopril (100 mg kg^{-1} day^{-1}, p.o.). An arrow indicates desmin-positive area. Original magnification ×400.

cells/glomerular cross-section vs. the normal control 40.1 ± 2.8 cells/glomerular cross-section, proliferating cell nuclear antigen-positive cells: the nephritic control 5.9 ± 0.8 cells/100 cells vs. the normal control 2.8 ± 0.4 cells/100 cells). In contrast, on day 40, KD3-671 at 10 mg/kg markedly suppressed the increase in the number of total cells (hypercellularity) and proliferating cells (proliferating cell nuclear antigen-positive cells) by 60% and 74%, re-

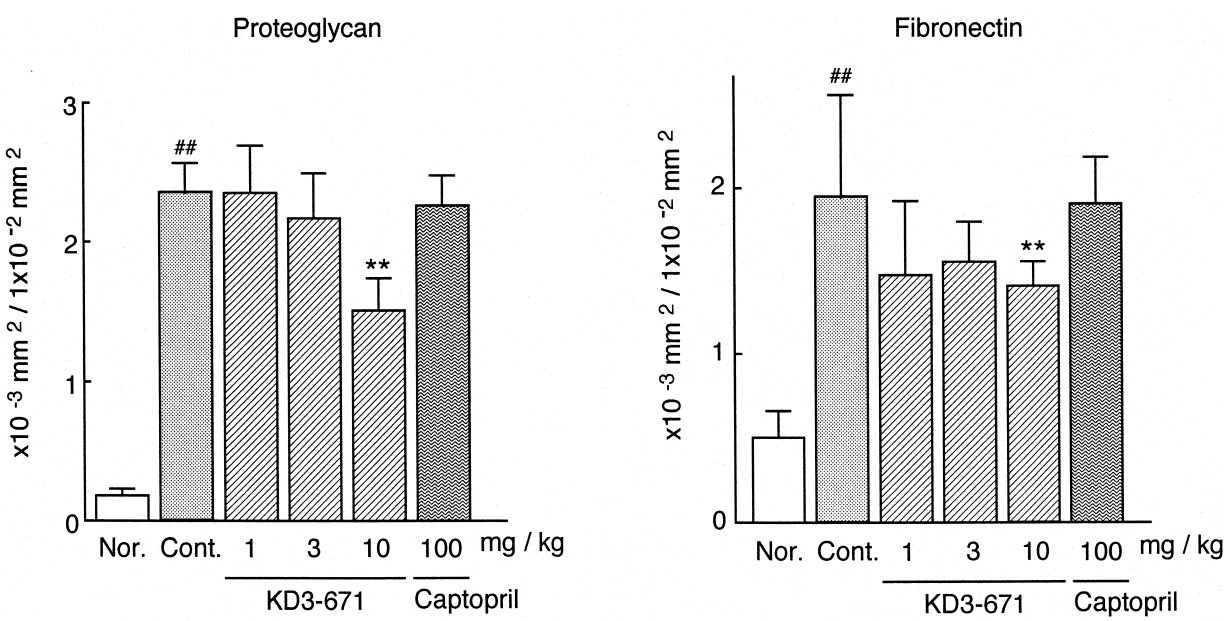

Fig. 8. Effect of KD3-671 on deposition of proteoglycan and fibronectin in the glomeruli of rats with anti-glomerular basement membrane antibody-associated glomerulonephritis. The deposition of proteoglycan and fibronectin in the glomeruli is expressed as $\times 10^{-3}$ $mm^2/1 \times 10^{-2}$ mm^2. Test drugs were given from day 1 after i.v. injection of anti-glomerular basement membrane serum. Kidneys were taken at day 40. $\#\#P < 0.01$, compared to the normal group. $^{**}P < 0.01$, compared to the nephritic control group.

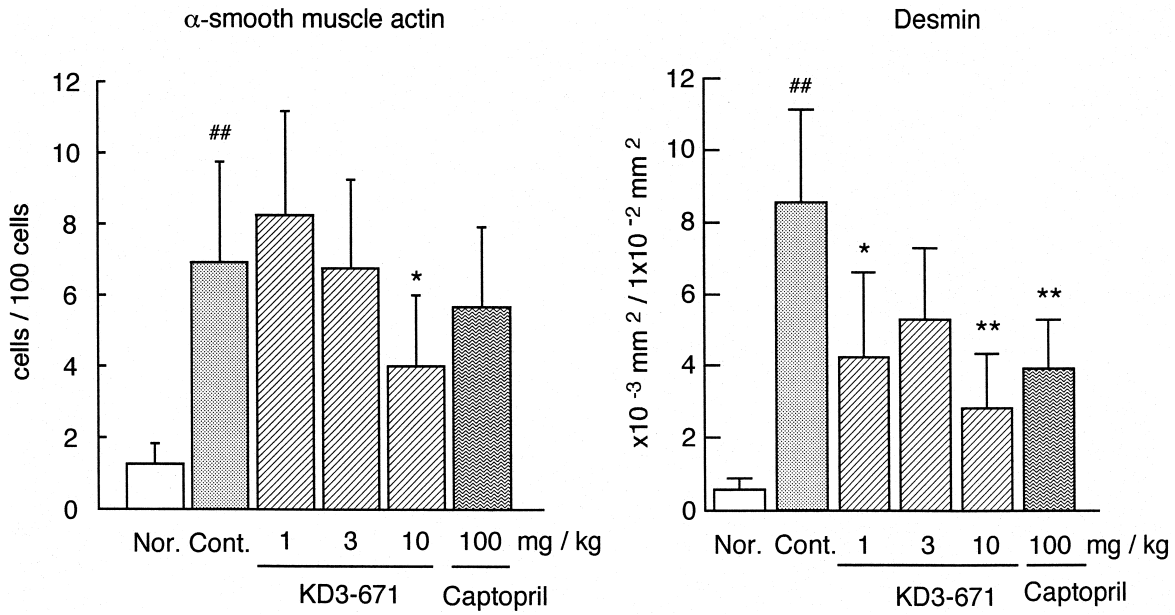

Fig. 9. Effect of KD3-671 on α-smooth muscle actin and desmin expression in the glomeruli of rats with anti-glomerular basement membrane antibody-associated glomerulonephritis. The number of α-smooth muscle actin-positive cells in the glomeruli is expressed as cells/100 cells. The desmin-positive area in the glomeruli is expressed as 10^{-3} $mm^2/1 \times 10^{-2}$ mm^2. Test drugs were given from day 1 after i.v. injection of anti-glomerular basement membrane serum. Kidneys were taken at day 40. $^{*}P < 0.05$ and $^{**}P < 0.01$, compared to the nephritic control group.

spectively. KD3-671 at 1 and 3 mg/kg and captopril were also effective to suppress the increase in the number of total cells.

3.3. The deposition of extracellular matrix protein and the number of transformed cells (Figs. 7–9)

In the normal kidneys, proteoglycan and fibronectin were seen in the mesangial matrix and along the glomerular basement membrane. The nephritic glomeruli showed increased deposition of proteoglycan and fibronectin in the mesangial area as well as thickening of capillary wall. In KD3-671- or captopril-treated rats, a significant diminution in the glomerular deposition of both extracellular matrix proteins was noted.

We also investigated the number of transformed cells in the nephritic glomeruli with monoclonal antibody against α-smooth muscle actin or desmin by an indirect immunoperoxidase technique. Administration of KD3-671 at 10 mg/kg resulted in fewer glomerular α-smooth muscle actin-positive cells and a smaller desmin-positive area. Captopril also suppressed the increase of the desmin-positive area by 58%.

3.4. Plasma antibody levels against rabbit-immunoglobulin G and deposition of rat-immunoglobulin G

The nephritic rats showed a markedly accelerated antibody production. The accelerated antibody production was not affected by KD3-671 or captopril. Rat-immunoglobulin G was deposited on the glomerular capillary lumen in the nephritic control rats. KD3-671 and captopril did not affect deposition of rat-immunoglobulin G in the glomeruli (data not shown).

4. Discussion

In the present studies, we demonstrated that KD3-671 suppressed the elevation of urinary protein (Fig. 2) and plasma urea nitrogen (Fig. 3), and the development of histopathological alterations in the glomeruli (Figs. 4 and 5). The efficacy of KD3-671 was similar to that of captopril in terms of proteinuria. It is well-established that hypertension accelerates the development of, and aggravates renal disease (Suzuki and Nagamatsu, 1978, Suzuki et al., 1986). Ots et al. (1998) observed that losartan, an angiotensin AT_1 receptor antagonist, suppressed the development of renal injury in rats subjected to 5/6 renal mass ablation, and considered that the antihypertensive effect was a crucial factor to prevent histological alterations. However, is the hypotensive effect of KD3-671 the most crucial factor for the antinephritic effect of this agent on the present experimental nephritis? Mochizuki et al. (1995) demonstrated that 3 mg/kg of KD3-671 reduced blood pressure from 180 mm Hg to 100 mm Hg in renal artery-ligated hypertensive rats. They also observed that 1 mg/kg of KD3-671 was a sufficient dose to decrease the blood pressure that had been elevated by the infusion of angiotensin II in rats (Mochizuki et al., 1995). On the other hand, in the present studies, 1 and 3 mg/kg of KD3-671 failed to suppress the progression of nephritis although 10

mg/kg of KD3-671 significantly attenuated the nephritis. Therefore, while we did not measure blood pressure during the experiments, we do not think that the antinephritic effect of KD3-671 results from a hypotensive effect. Moreover, captopril suppressed urinary protein excretion even in normotensive diabetic nephropathy patients (Mimran et al., 1988). Thus, we speculate as follows regarding the possible mechanisms for the antinephritic effect of KD3-671, in addition to its hypotensive effect.

The increase in cell counts in the nephritic glomeruli is due to cell proliferation and cell infiltration as observed in the present studies. Cell proliferation in the glomeruli is a major histological feature of various glomerular diseases, including anti-glomerular basement membrane antibody-associated glomerulonephritis in humans (Rosen, 1983). KD3-671 markedly suppressed the increase in proliferating cell nuclear antigen-positive cells and hypercellularity in the nephritic glomeruli (Figs. 4 and 6). These findings may be partially accounted for by the evidence that angiotensin II exerts a mitogenic effect on cultured mesangial cells, and that an angiotensin AT_1 receptor antagonist inhibits it (Ray et al., 1991; Wolf and Neilson, 1993). Additionally, leukocytes are observed in the glomeruli in anti-glomerular basement membrane antibody-associated nephritis (Hattori et al., 1994). Because angiotensin AT_1 receptor antagonists abolish both glomerular monocyte chemoattractant protein-1 expression and macrophages/monocytes in anti-thymocyte serum-induced nephritis (Wolf et al., 1998), KD3-671 may decrease cell counts through the suppression of monocyte chemoattractant protein-1 expression in the nephritic model (Grandaliano et al., 1994; Rovin et al., 1994; Wenzel et al., 1997).

Furthermore, when an angiotensin-converting enzyme inhibitor or hidralazine was administered to Tukuba hypertensive mice, which are transgenic mice that carry human renin and angiotensinogen genes and develop hypertension and glomerulosclerosis, both agents significantly reduced blood pressure and protected the kidneys from glomerulosclerosis (Kai et al., 1998). Interestingly the angiotensin-converting enzyme inhibitor inhibited the expression of fibronectin in the glomeruli, but hydralazine did not. These finding are consistent with the present results indicating that fibronectin expression in the glomeruli with anti-glomerular basement membrane antibody-associated nephritis was attenuated by the treatment of KD3-671 (Figs. 7 and 8). While it is well known that fibronectin plays an important role in wound healing, an excessive expression of fibronectin leads to crescent formation and fibrosis in the glomeruli of the experimental nephritic animals (Sady et al., 1995). KD3-671 also suppressed the excessive expression of proteoglycan in the nephritic glomeruli (Figs. 7 and 8). Angiotensin II stimulates the production of plasminogen activator inhibitor-1, suggesting that angiotensin II contributes to the persistence of fibrin deposits and extracellular matrix accumulation in the nephritic glomeruli (Wilson et al., 1997). TGF-β is a multifunctional cytokine that is considered to play a major biological role in regulating the production of extracellular matrix protein (Border and Ruoslahti, 1990; Roberts et al., 1992). TGF-β gene expression increases in several models of acute and chronic renal injury in parallel with extracellular matrix protein production (Ketteler et al., 1994; Ruiz-Ortega et al., 1995). TGF-β up-regulates the synthesis of fibronectin in mesangial cells (Border et al., 1990). An excessive expression of fibronectin is considered to lead to crescent formation and fibrosis in the glomeruli in anti-glomerular basement membrane antibody-associated glomerulonephritis (Sady et al., 1995). Recent studies further shown the interaction of angiotensin II and TGF-β. Angiotensin II induces the expression of TGF-β mRNA and results in an increase of TGF-β expression in the glomeruli, which in turn induces fibrotic changes (Kagami et al., 1994). Expression of TGF-β mRNA is up-regulated in the glomeruli of experimental nephritic models, and an angiotensin AT_1 receptor antagonist suppresses it (Yayama et al., 1995b; Zoja et al., 1998). Moreover, TGF-β-producing cells are resident glomerular cells that express α-smooth muscle actin and/or desmin (Kagami et al., 1994). In the current investigation, KD3-671 inhibited the expression of α-smooth muscle actin and desmin (Figs. 7 and 9) and the production of fibronectin and proteoglycan in the nephritic glomeruli (Figs. 7 and 8). Angiotensin II also contributes to the persistence of fibrin deposits and extracellular matrix accumulation in addition to increasing production of extracellular matrix in the nephritic glomeruli (Wilson et al., 1997). Thus KD3-671 may inhibit the synthesis of TGF-β although the precise mechanisms are unknown.

In summary, we demonstrated that KD3-671 suppressed the progression of anti-glomerular basement membrane antibody-associated glomerulonephritis and discussed putative mechanisms of KD3-671 action. This is the first investigation to reveal the anti-nephritic effect of KD3-671 in this experimental model of nephritis. Work is in progress to evaluate the therapeutic benefits of KD3-671 in the established phase of this nephritic model.

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