TJN-259 Improves Mesangial Lesions in Experimental Immunoglobulin A Nephropathy in ddY Mice

Chiharu SADAKANE,[a,b] Tomohisa HATTORI,*[,a] Junichi KOSEKI,[a] Yayoi INAGAKI,[a,b] Yoshihiro HASEGAWA,[a] Shoichiro SHINDO,[a] Shuichi TAKEDA,[a] and Hiroshi TAKEDA[b]

[a] *Tsumura Research Laboratories, Tsumura & Co.; 3586 Yoshiwara, Ami-machi, Inashiki-gun, Ibaraki 300–1192, Japan: and* [b] *Department of Pathophysiology and Therapeutics, Division of Pharmasciences, Faculty of Pharmaceutical Sciences, Hokkaido University; N12 W6, Kita-ku, Sapporo 060–0812, Japan.* Received June 25, 2009; accepted July 8, 2009

TJN-259 is a chemical substance based on the structural features of the botanically derived ingredient acteoside. This study was performed in order to elucidate the antinephritic effects of TJN-259 in experimental immunoglobulin A (IgA) nephropathy. In this study, 28-week-old ddY mice were used as a spontaneous model of IgA nephropathy. With regard to spontaneous IgA nephropathy, we investigated the effects of TJN-259 administered from 28 to 40 weeks. In addition, an accelerated model of IgA nephropathy was experimentally induced in ddY mice by oral administration of bovine serum albumin, followed by reticuloendothelial blocking by colloidal carbon injection and heminephrectomy. At 10 weeks after the 3rd carbon injection, we also examined the effects of TJN-259 on accelerated IgA nephropathy. To investigate the effects of TJN-259 on transforming growth factor (TGF)-β1 production in accelerated IgA nephropathy, kidneys were isolated and measured TGF-β1 by the enzyme-linked immunosorbent assay (ELISA) method. The administration of TJN-259 to mice with spontaneous IgA nephropathy decreased the incidence of mesangial expansion as well as the number of nuclei per glomerular cross-section in comparison with that of non-treated mice. In addition, TJN-259 treatment prevented the increase in the incidence of mesangial expansion, crescent formation, and segmental sclerosis in glomeruli in accelerated IgA nephropathy. TJN-259 also inhibited the increased immunostaining score of collagen type IV and TGF-β1 in glomeruli of accelerated IgA nephropathy. Treatment with TJN-259 inhibited the increases in renal total and mature TGF-β1 protein levels in accelerated type IgA nephropathy. TJN-259 failed to inhibit the increase in serum IgA levels in both models. These results suggest that TJN-259 was an effective treatment against IgA nephropathy in ddY mice, acting *via* the suppression of TGF-β1 production in glomeruli.

Key words immunoglobulin nephropathy; TJN-259; transforming growth factor-β1; mouse

Proliferation of renal mesangial cells is a typical glomerular pathological finding in renal diseases, and it is observed in most proliferative nephritises (including immunoglobulin A (IgA) nephropathy). The mechanism of action of the expansion of the glomerular mesangial region, a particularly notable feature of IgA nephropathy, is not well elucidated, but it is presumed that the inflammatory reactions after deposition of IgA play a critical role. Spontaneous nephropathy models employing HIGA[1)] or ddY mice[2)] are used as experimental animal models of IgA nephropathy. Analyses of the effects of chemical compounds against IgA nephropathy have been conducted using these models; however, such analyses face an inherent difficulty in identifying the dramatic changes that occur in the inflammatory process because it takes a long time for the models to develop the disease. Until the present time, there have thus been no revolutionary therapeutic agents against IgA nephropathy. On the other hand, unilateral nephrectomy, blockade of the reticuloendothelial system, and oral sensitization in ddY mice have demonstrated that the deposition of IgA and pathological changes in glomeruli may be induced earlier than those in spontaneous ddY mice.[3—5)]

Transforming growth factor-β1 (TGF-β1) induces matrix synthesis in renal tissues, leading to the progression of IgA nephropathy.[6,7)] When added to cultured human mesangial cells, aggregated IgA1 from patients with IgA nephropathy induces increases in TGF-β1 gene expression and secretion of extracellular matrix in cultured mesangial cells.[8)] These findings suggest that TGF-β1 is a therapeutic target for human IgA nephropathy.

Acteoside, which is found in *Stachys sieboldii* MIQ., dramatically improves urinary protein excretion and histopathological changes in rat anti-glomerular basement membrane (GBM) nephritis and also inhibits mesangial cell expansion in anti-Thy1 nephritis.[9—11)] Acteoside is an herbal drug component[9)] that has a structure composed of caffeic acid and phenethyl alcohol bridged by two types of sugar moieties. By screening a series of compounds with pyridyl acrylic amide backbones that were chemically derived from caffeic acid and phenethyl alcohol for potency of antinephritic action in an *in vivo* assay, a new compound known as TJN-259 was generated. However, the detailed mechanisms of its action have not yet been elucidated.

In this study, we investigated the therapeutic effects of TJN-259 on early glomerular lesions in a spontaneous model of IgA deposition and an accelerated model of IgA nephropathy in ddY mice in order to elucidate the effects of TJN-259 on IgA nephropathy.

MATERIALS AND METHODS

Chemicals TJN-259 (M.W. 354.4), one of (pyridyl)-acrylic acid derivatives was synthesized and derived by Tsumura & Co. (Tokyo Japan) (Fig. 1).[12)] Captopril and

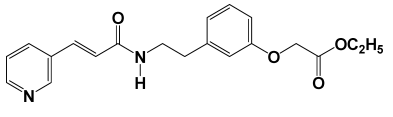

Fig. 1. Chemical Structure of TJN-259

* To whom correspondence should be addressed. e-mail: hattori_tomohisa@mail.tsumura.co.jp

dipyridamole (SIGMA-Aldrich, St. Louis, MO, U.S.A.) were used as the other test articles. All test articles were prepared in a 0.5% carboxymethylcellulose suspension (CMC). A rabbit TGF-β1 antibody (Genzyme Co., MA, U.S.A.) and a rabbit collagen type IV antibody (Chemicon Co., MA, U.S.A.) were used for immunohistochemistry.

Induction of the Experimental Model Spontaneous model of IgA nephropathy: Twenty-eight week old male ddY mice (Japan SLC, Inc., Shizuoka, Japan) were used. The mice were divided into six groups ($n=10$) so serum IgA levels were comparable among the groups. After being grouped, the mice were administered TJN-259 (0.4, 2.0, or 10 mg/kg/d) or captopril (10 mg/kg/d), orally using a metal orogastric tube until the mice were 40 weeks of age. At 40 weeks old, blood collection was conducted, along with removal of the left kidney for visual inspection using an optical microscope. Accelerated model of IgA nephropathy: Five-week-old male ddY mice (Japan SLC, Inc.) were used. The mice were habituated for 1 week and then used for experiments. Induction of an accelerated model of IgA nephropathy was performed using the method reported by Sato *et al.*[3,5] The right abdominal region was opened under ether anesthesia, and the right renal artery was ligated using suture thread to occlude the right kidney. After one week, colloidal carbon (Pilot Co., Tokyo, Japan) suspended in a 1% gelatin solution to block the reticuloendothelial system was administered intravenously to the mouse caudal vein at a dosage of 4 mg/100 g body weight. This carbon suspension was administered to mice at 1-week intervals for a total of 3 administrations. Bovine gamma globulin (Seikagaku Co., Tokyo, Japan) was added to the drinking water at a dosage of 1 g/l upon the first carbon administration, and free ingestion was permitted during the entire experiment. At 10 weeks after the 3rd carbon administration, the mice were grouped into 7 groups ($n=8$). TJN-259 (0.4, 2.0, or 10 mg/kg/d) or captopril (10 mg/kg/d) was orally administered to each group for 5 weeks beginning just after the grouping and lasting until 15 weeks. Non-treated ddY mice or control mice were administered 0.5% CMC as a solvent. Blood collection was conducted, along with removal of the left kidney for visual inspection using an optical microscope. All experimental protocols were conducted in accordance with the guidelines of Tsumura & Co. (Ibaraki Japan) for the care and use of laboratory animals.

Measurement of Proteinuria, Serum Creatinine and Blood Urea Nitrogen (BUN) Urine was collected and centrifuged. The supernatant was collected as a sample for proteinuria (Tonein-TP II, Otsuka Pharmaceutical Co., Ltd., Tokyo Japan). Blood was centrifuged, and the supernatant was collected as a sample for serum creatinine and IgA level determination. The serum creatinine or BUN level was evaluated using a Toshiba (Tokyo, Japan) model TBA 20FR automatic analyzer.

Serum IgA Determination Serum IgA levels were measured using an enzyme-linked immunosorbent assay quantitation kit (Bethyl Laboratories Montgomery, TX, U.S.A.). Specific IgAs against bovine γ globulin were detected by enzyme-linked immunosorbent assay (ELISA). Standard and 1000-fold dilution samples were coated on 96 well plates at 4 °C overnight, and then the samples were added and incubated for 2 h. A peroxide-conjugated anti-mouse IgA antibody (Cappel Co., MA, U.S.A.) was applied to each well and the well was washed three times. The substrate was added to the well and we then measured the wavelength at 490 nm using a multi-plate reader (Bio-tek instruments FL312e BIO–KINETICS READER).

Histopathological Evaluation The harvested kidneys were fixed in Methyl Carnoy's solution and embedded in paraffin. Tissue sections of 3 μm were prepared and stained with periodic acid methenamine silver (PAM) and periodic acid-Schiff (PAS). The number of nuclei per glomerular cross-section and the incidence of mesangial expansion, crescent formation, and segmental sclerosis were determined in a blind manner with 50 randomly selected glomeruli that included the vascular pole. The immunostaining scores were assigned based on the extent of glomerular staining according to the modified method reported by Kagami *et. al.*[13]:

0 points, no glomerular staining
1 point, weak glomerular staining
2 points, segmental staining
3 points, global staining (below 50%)
4 points, global staining (more than 50%)
Score=[$\sum$ (each score$\times$number of glomeruli)]/50

Effect of TJN-259 on TGF-β1 Production Next, we examined the direct effects of TJN-259 on renal TGF-β1 production in an accelerated model of IgA nephropathy in mice. Accelerated IgA nephropathy was induced by the above method. After the 3rd carbon administration, mice were grouped into 9 groups ($n=8$). TJN-259 (2 mg/kg/d) was orally administered to each group for 1 d, 3 d, and 7 d. Non-treated controls were given 0.5% CMC as a control. Kidneys were harvested 1 h after the last administration of the test drugs on days 1, 3, and 7. Isolated kidneys were homogenized with 3.0 ml phosphate buffered saline (PBS) and then centrifuged at 3000 rpm for 10 min. Total or mature TGF-β1 levels in each supernatant were detected by an ELISA (Amersham Pharmacia Biotech, MA, U.S.A.).

Statistical Analyses All data were expressed as the mean$\pm$S.E.M. Statistical significance was measured by a one-way analysis of variance (ANOVA) followed by Dunnett analysis. Significance was accepted at $p<0.05$.

RESULTS

Effect on the Spontaneous Model of IgA Nephropathy General Condition and Body Weight (Data Not Shown in Figures and Tables): No mice given TJN-259 died during the experimental period. A similar amount of mice chow was consumed by both the nontreated mice and test drug-treated mice. Both nontreated mice and TJN-259-treated mice showed a similar course of body weight gain.

Effects on Serum Creatinine or Serum IgA Levels (Data Not Shown in Figures and Tables): Serum creatinine levels in 40-week-old ddY mice were not significantly increased compared with the levels in 27-week-old animals. Each value is within the normal range. Serum IgA levels in control mice at 40 weeks were markedly increased compared with those in 28-week-old ddY mice (approximately 0.8 μg/ml). Each test drug showed a tendency to decrease serum IgA levels at 40 weeks.

Histopathological Evaluation Effects on Expansion of

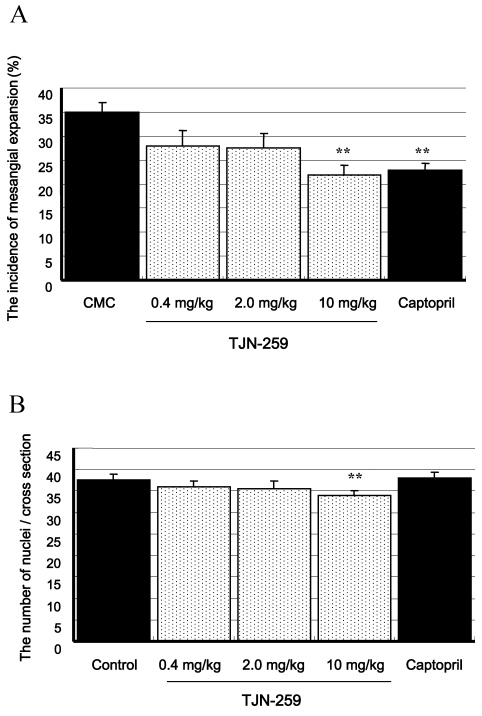

Fig. 2. Effects of TJN-259 on the Incidence of Mesangial Expansion (A) and the Number of Nuclei Per Cross-Section (B) in the Glomeruli of Spontaneous IgA Nephropathy Mice

Each bar represents the mean±S.E.M. of 10 mice. The mice were administered TJN-259 at doses of 0.4, 2.0 and 10 mg/kg/d or captopril, *p.o.*, at a dose of 10 mg/kg/d *p.o.* from 28 to 40 weeks. The kidney was removed 1 h after the last administration of test drugs at 40 weeks. Group means significantly different from the control (CMC-treated group) are marked; ** $p<0.01$ *vs.* control, Dunnett's test.

the Mesangial Region: Control ddY mice at 40 weeks displayed mild mesangial expansion, and such expansion reached levels of 35% in glomeruli (Fig. 2A). In contrast, treatment with TJN-259 (10 mg/kg/d) and captopril significantly inhibited mesangial expansion. Representative micrographs of glomeruli from control mice and TJN-259-treated mice are shown in Fig. 3.

Effects on the Number of Nuclei in Glomerular Cross Sections: The number of nuclei in 40-week-old ddY mice was approximately 38 nuclei/cross-section. TJN-259 treatment (10 mg/kg/d) significantly reduced the number of nuclei per cross-section at 40 weeks (Fig. 2B). Captopril failed to reduce this parameter.

Effects on TGF-β1 Staining Score (Data Not Shown): The glomeruli observed at 40 weeks showed only trace levels of TGF-β1 staining in this study. Therefore, we could not properly evaluate this parameter.

Effects on the Accelerated IgA Nephropathy Model General Condition and Body Weight (Data Not Shown in Figures and Tables): The nontreated mice with IgA nephropathy and test drug-treated mice had a tendency to be lower body weight than that of the non-treated mice during the experimental period. No mice given TJN-259 or other test drug died during the experimental period. A similar amount of mice chow was consumed by both the nontreated IgA nephropathy mice and TJN-259 or other test drug treated mice. Both nontreated mice and TJN-259 or other test drug-treated mice showed a similar course of body weight gain.

Effects on Proteinuria, Serum Creatinine and BUN Levels: Proteinuria in accelerated IgA nephropathy did not increase, compared with non-treated ddY mice during the experimental period. Serum creatinine levels in the accelerated model of IgA nephropathy were 0.75±0.05 mg/dl. Administration of TJN-259 significantly inhibited the elevation of serum creatinine levels in the accelerated model of IgA nephropathy. Captopril also inhibited the increase in serum creatinine levels (Fig. 4). BUN level in non-treated mice was 34.7± 0.9 mg/dl. In addition, BUN level in mice with IgA nephropathy (control) (38.6±2.4 mg/dl) was not significantly enhanced compared with non-treated group.

Effects on Serum IgA Level (Data not Shown in Figures and Tables): Levels of serum-specific IgA against bovine γ globulin in the IgA nephropathy mice at 15 weeks were markedly increased compared with those in non-treated mice. TJN-259 at a dose of 2.0 mg/kg/d showed a tendency to inhibit the elevation of serum IgA levels. Captopril did not affect the serum IgA levels. In this study, the nonspecific serum IgA levels in mice with IgA nephropathy did not show any significant change in comparison with levels in non-treated mice.

Histopathological Evaluation: i) Effects on the Expansion of the Mesangial Region: Figure 5 shows representative light microscopic pictures at the end of experimental period. Control mice displayed moderate mesangial expansion, with an incidence of 80% in the observed glomeruli (Fig. 6A). In contrast, treatment with TJN-259 at doses of 1.0 or 2.0 mg/kg/d significantly inhibited mesangial expansion. Captopril failed to inhibit mesangial expansion.

ii) Effects on Crescent Formation in Glomeruli: The incidence of crescent formation in control mice was approximately 12% in 50 observed glomeruli. Administration of TJN-259 significantly inhibited crescent formation, and captopril showed a tendency to inhibit crescent formation (Fig. 6B).

iii) Effects on Segmental Sclerosis in Glomeruli: The incidence of segmental sclerosis in glomeruli was approximately 25% in 50 observed glomeruli. TJN-259 treatment at a dose of 2.0 mg/kg/d significantly inhibited segmental sclerosis. Captopril showed a tendency to inhibit segmental sclerosis (Fig. 6C).

iv) Effects on the Glomerular Collagen Type IV and TGF-β1 Staining Score: Figure 7 shows a representative immunostaining area of TGF-β1 at the end of experimental period. The glomerular collagen type IV staining score in control mice was significantly greater than that in non-treated mice. TJN-259 at a dose of 2.0 mg/kg inhibited the increase in the collagen type IV staining score. Captopril did not affect this parameter (Fig. 8). The glomerular TGF-β1 staining score in control mice was markedly increased compared with that in non-treated mice. Administration of TJN-259 at a dose of 2.0 mg/kg significantly inhibited the increase in the glomerular TGF-β1 staining score. Captopril showed a tendency to inhibit this score (Fig. 8).

Effects on TGF-β1 Production: Total and mature renal TGF-β1 levels in mice with accelerated IgA nephropathy were markedly increased during the experimental periods in comparison with non-treated mice. Administration of TJN-259 significantly inhibited the increase in total TGF-β1 levels on the 1st and 3rd days. The increase in the mature renal TGF-β1 levels was markedly reduced by TJN-259 treatment

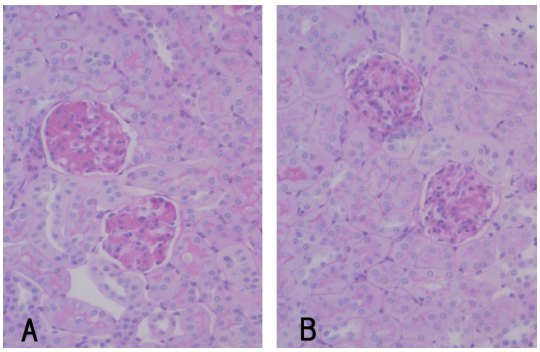

Fig. 3. Periodic Acid Schiff Staining of Tissue Obtained from Spontaneous IgA Nephropathy in Mice

(A) Mice with IgA nephropathy given 0.5% CMC; (B) mice with IgA nephropathy given TJN-259 at 10 mg/kg/d, *p.o.* (×440).

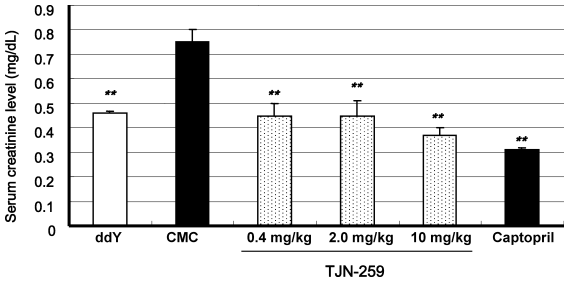

Fig. 4. Effects of TJN-259 on Serum Creatinine Level in Accelerated IgA Nephropathy

The mice were administered TJN-259 at doses of 0.4, 2.0 and 10 mg/kg/d, *p.o.* or captopril, *p.o.*, at a dose of 10 mg/kg/d, *p.o.* from 10 weeks to 15 weeks. Blood was collected 1 h after the last administration of test drug at 15 weeks. Group means significantly different from the control are marked; $**p<0.01$ *vs.* control; Dunnett's test. Each bar represents the mean±S.E.M. of 9 mice.

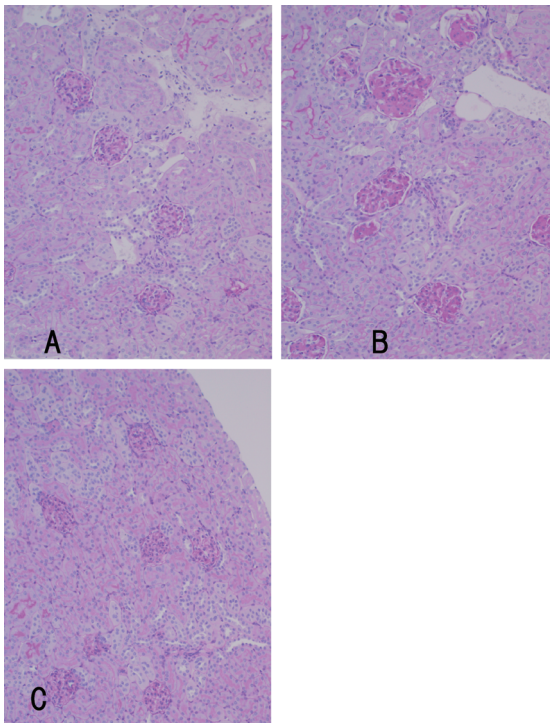

Fig. 5. Periodic Acid Schiff Staining of Tissue Obtained from Accelerated IgA Nephropathy in Mice

(A) Non-treated ddY mice; (B) mice with IgA nephropathy given 0.5% CMC; (C) mice with IgA nephropathy given TJN-259 at 10 mg/kg/d, *p.o.* (×220).

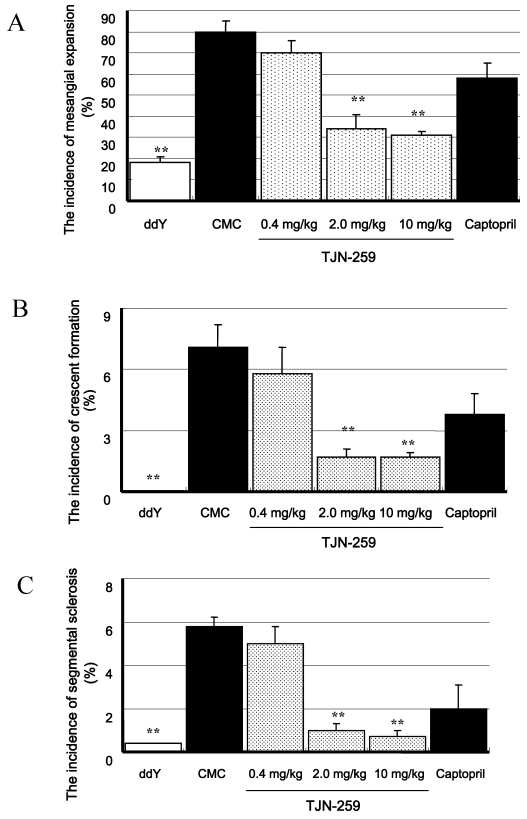

Fig. 6. Effects of TJN-259 on the Increased Incidence of Mesangial Expansion, Crescent Formation and Segmental Sclerosis in Accelerated IgA Nephropathy

The mice were administered TJN-259 at doses of 0.4, 2.0 and 10 mg/kg/d, *p.o.* or captopril, *p.o.*, at a dose of 10 mg/kg/d, *p.o.* from 10 to 15 weeks. The kidney was removed 1 h after the last administration of the test drug at 15 weeks. Group means significantly different from the control are marked; $**p<0.01$ *vs.* control; Dunnett's test. Each bar represents the mean±S.E.M. of 9 mice.

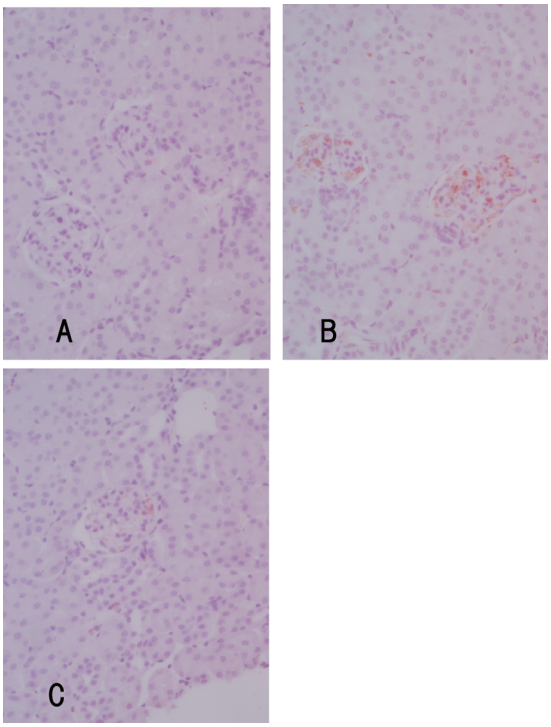

Fig. 7. Glomerular Immunostaining for TGF-β1

(A) Non-treated ddY mice, (B) mice with IgA nephropathy given 0.5% CMC, (C) mice with IgA nephropathy given TJN-259 10 mg/kg/d, *p.o.*

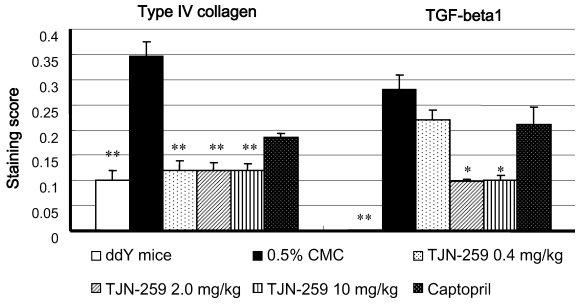

Fig. 8. Effects of TJN-259 on the Increase in the Glomerular Collagen Type IV or TGF-β1 Staining Score in Accelerated IgA Nephropathy

The mice were administered TJN-259 at doses of 0.4, 2.0 and 10 mg/kg/d captopril, *p.o.*, at a dose of 10 mg/kg/d *p.o.* or dipyridamole at a dose of 200 mg/kg/d *p.o.* from 10 to 15 weeks. The kidney was removed 1 h after the last administration of the test drug at 40 weeks. Group means significantly different from the control are marked; *, ** $p<0.05$, 0.01 *vs.* control (0.5% CMC-treated group); Dunnett's test. Each bar represents the mean±S.E.M. of nine mice.

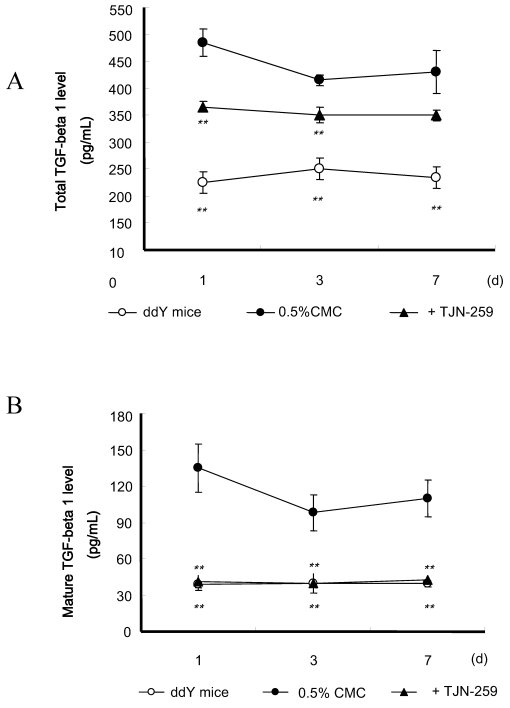

Fig. 9. Effects of TJN-259 on Total and Mature Renal TGF-β1 Levels in Accelerated IgA Nephropathy

The mice were divided into 9 groups such that the mean body weight and standard error were approximately the same for all groups on the day after the last carbon injection. The mice were administered TJN-259 at doses of 0.4, 2.0 and 10 mg/kg/d *p.o.* The kidney was removed on the 1st, 3rd and 7th days after the last carbon injection. Group means significantly different from the control are marked; *, ** $p<0.05$, 0.01 *vs.* control; Dunnett's test. Each bar represents the mean±S.E.M. of 9 mice. (A) Total renal TGF-β1 levels in accelerated IgA nephropathy. (B) Mature renal TGF-β1 levels in accelerated IgA nephropathy. The levels of mature TGF-β1 were measured by adding 1 mol/l HCl to the kidney homogenates.

(Fig. 9).

DISCUSSION

The present study demonstrates that administration of TJN-259 inhibited the glomerular histopathological changes in both models of IgA nephropathy. In addition, TJN-259 prevented the increase in both the TGF-β1 staining score in glomeruli and the renal TGF-β1 protein levels in the accelerated model of IgA nephropathy. Interestingly, the inhibitory actions of TJN-259 against mature TGF-β1 levels were stronger than those against total TGF-β1 levels. It is likely that TJN-259 may inhibit the activity of an unknown protease through which TGF-β1 converts from a latent to an active form. We speculate that TJN-259 may prevent the increased accumulation of mesangial matrix components, such as collagen type IV, *via* the inhibition of the conversion of TGF-β1 to its mature form in glomeruli.

Recently, it has been demonstrated in human IgA nephropathy that TGF-β1 plays important roles in the progression of the disease.[6—8] Chihara *et al.*[6] reported that the level of TGF-β1 gene expression in renal biopsies in human IgA nephropathy was significantly correlated with histopathological damage (*e.g.*, sclerosis of glomeruli). Moreover, a positive correlation between urinary protein excretion levels and TGF-β1 mRNA levels has been demonstrated in renal biopsies from patients with IgA nephropathy.[7] We previously reported that the level of renal TGF-β1 protein was significantly increased in experimental nephritic mice. Additionally, the administration of a neutralizing antibody against TGF-β1 inhibited mesangial matrix expansion.[14] Although not shown in this study, preliminary study demonstrated that the treatment of accelerated IgA nephropathy mice with a TGF-β1 neutralizing antibody for 1 week after the induction of nephropathy ameliorates the decrease in creatinine clearance (non-treated ddY mice: 10.0± 0.75 ml/min, unilateral nephrectomized IgA nephropathy: 1.8±1.5 ml/min, +TGF-β1 neutralizing antibody: 12.4± 1.5 ml/min, $p<0.01$ *vs.* mice with IgA nephropathy, Student's *t*-test) as well as the increase in the mesangial expansion score (non-treated ddY mice: 0.05±0.01, unilateral nephrectomized IgA nephropathy: 0.6±0.08, +TGF-β1 neutralizing antibody: 0.28±0.03, $p<0.01$ *vs.* mice with IgA nephropathy, Dunnett's analysis. In this study, we could not detect glomerular TGF-β1 staining using immunohistochemistry in preliminary study. In the early stages of the accelerated model of IgA nephropathy in which could not observe glomerular TGF-β1 expression, TGF-β1 may be a key factor leading to the deterioration of renal function.

TJN-259 prevented mesangial expansion and the increased number of nuclei in glomeruli with spontaneous IgA nephropathy, although TGF-β1 staining score in glomeruli showed a trace as seen in early stage in accelerated IgA nephropathy. We can not explain this phenomenon as yet. Oyama *et al.*[15] reported that T or B cells from mice with spontaneous IgA nephropathy exhibited higher levels of expression of TGF-β1 and TGF-β1 mRNA than BALB/c mice at 40 weeks age. TGF-β1 is one of the factors that stimulate B cell class switching to surface IgA-positive cells, and also enhances the accumulation of extracellular matrix components. Indeed, we had not measured TGF-β1 level in splenic mononuclear cell. It is considered likely that the increased TGF-β1 expression in mononuclear cells or intestinal Peyer's patches might be observed in our models and administration of TJN-259 prevented it.

Recently, it has been demonstrated that cultured glomerular mesangial cells express an Fc receptor for IgA and IgG. Further, IgA–IgG complexes caused an augmentation of TGF-β1 mRNA levels as well as the conversion of latent TGF-β1 to a biologically active form in mesangial cells.[16] The present data indicate that TJN-259 treatment failed to in-

hibit the enhancement of serum IgA levels in both models. This means probably due to the possible mechanisms by which TJN-259 may act locally to markedly increase the production of TGF-β1 after the IgA complex accumulation in glomeruli. In order to elucidate the direct effects of TJN-259 on renal TGF-β1 production, we next investigated the effects of TJN-259 on TGF-β1 protein derived from renal tissue on the 1st, 3rd, and 7th days after the induction of an accelerated model of IgA nephropathy. On the 1st day after the induction of nephropathy, renal TGF-β1 levels were markedly increased; a single administration of TJN-259 inhibited the increase of both total and mature TGF-β1 protein. Notably, TJN-259 strongly inhibited the increase in mature TGF-β1 levels throughout the experimental period. In addition, we preliminarily evaluated the effects of TJN-259 on TGF-β1 production from murine anti-GBM nephritic glomeruli in an *in vitro* assay. TJN-259 at a dose of 50 μmol/l inhibited the production of total TGF-β1 by 38%, and it also inhibited the conversion from a latent to active form by 57.3%. Thus, these findings suggest that TJN-259 directly inhibits TGF-β1 maturation in inflamed renal tissue.

This study evaluated the spontaneous model of IgA nephropathy as well as the accelerated model, in which ddY mice underwent a unilateral nephrectomy. The reticuloendothelial system was blocked by carbon injection and oral administration of bovine γ globulin, which resulted in the acceleration of deposition of IgA in glomeruli. Glomerular TGF-β1 expression in 40-week-old ddY mice with spontaneous IgA nephropathy reached only trace levels, and the degree of histopathological change in the glomeruli was mild. On the other hand, we could detect a marked increase in TGF-β1 protein on the 1st day after the induction of the accelerated model. Although we could not detect TGF-β1 immunoreactivity at this early stage in the accelerated model of IgA nephropathy, the degree of staining for TGF-β1 in this stage may be mild, as in the spontaneous model. Therefore, we suggest that the sensitivity of our method to detect TGF-β1 immunoreactivity may be too low to observe TGF-β1 production in models of IgA nephropathy. It is likely that TJN-259 prevented histopathological changes in mice with both models of IgA nephropathy via the inhibition of TGF-β1 synthesis.

Heminephrectomy causes glomerular expression of TGF-β1 and extracellular matrix proteins, which leads to the progression of glomerulosclerosis.[4] Glomerular angiotensin convertase enzyme (ACE) in unilateral nephrectomized mice was significantly enhanced, and this enhancement suggests that the increase in the intraglomerular blood pressure might be caused by the progression of renal damage.[4] However, this study showed that captopril, a representative ACE inhibitor, failed to improve histopathological changes in an accelerated model of IgA nephropathy. Thus, the results suggest that the progression of a model combining unilateral nephrectomy, reticuloendothelial system blockade, and oral administration of bovine γ globulin may not depend on intraglomerular hypertension. One interesting possibility is that the blockade of the reticuloendothelial system and oral administration of protein in ddY mice, in addition to heminephrectomy, may be associated with increased glomerular IgA deposition; this would lead to TGF-β1 overproduction rather than hypertension.

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