The Effect of 6-Amidino-2-Naphtyl-4-Guanidinobenzoate Dimethane Sulfonate (FUT-175) on Experimental Glomerulonephritis in Mice

Hiroichi NAGAI, Hiroaki YAMADA, Naosuke MATSUURA, Naoki INAGAKI, Tsukasa SHIMAZAWA* and Akihide KODA
Department of Pharmacology, Gifu College of Pharmacy, 6-1 Mitahora-higashi, 5 chome, Gifu 502, Japan
*International Academy of Paramedical Technology, 759 Ichigahara aza Nagamine, Seki 501-32, Japan
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Abstract—Effect of 6-amidino-2-naphtyl-4-guanidinobenzoate dimethane sulfonate (FUT-175) on experimental glomerulonephritis in mice was studied. Employed models are nephrotoxic serum (NTS) nephritis in ddY or A/He mice, rabbit IgG (RGG) accelerated NTS nephritis in ddY mice and spontaneous nephritis in (NZB x NZW) F₁ mice. The severity of nephritis was evaluated by measuring proteinuria and serological parameters and examining renal tissue by light microscopy. Therapy with FUT-175 clearly prevented the pathological changes of proteinuria and serological parameters in all four nephritis models. By contrast, treatment hardly affected histopathological changes of the kidney in any of the models. Cyclophosphamide used as a comparative drug showed more clearly remission of the onset and development of NTS nephritis and RGG accelerated NTS nephritis in ddY mice by means of the changes of urinary and serological parameters. These evidences suggest that FUT-175 shows beneficial effects on the nephritis in either normal or complement deficient mice.

6-Amidino-2-naphtyl-4-guanidinobenzoate dimethane sulfonate (FUT-175) is reported to be a potent and reversible new synthetic complement inhibitor (1). In our previous studies on anti-allergic agents, we examined the effect of certain anti-complement agents on experimental allergic reactions and glomerulonephritis and described their efficacy (2, 3). The results suggested the possibility that anti-complement agent can serve as a remedy for glomerulonephritis. The present study was undertaken to investigate the effect of FUT-175 on experimental glomerulonephritis in mice with comparison to that of cyclophosphamide (Cy), one of the typical remedies for human glomerulonephritis.

Materials and Methods
Drugs: 6-Amidino-2-naphtyl-4-guanidinobenzoate dimethane sulfonate (FUT-175) was a gift from the Research Laboratories of Torii Co., Ltd., Tokyo, Japan. Cyclophosphamide (Cy) was purchased from Shionogi Pharmaceutical Co., Ltd., Osaka, Japan.

Animals: Male ddY and A/He mice weighing 18 to 20 g were used. The mice were purchased from Shizuoka Laboratory Animal Center. Female (NZB × NZW) F₁ mice weighing 18 to 20 g were kindly given by Nippon Shinyaku Co., Ltd., Kyoto, Japan. Food and water were provided ad libitum.

Nephrotoxic serum (NTS) nephritis in mice: NTS was prepared according to the method previously described (4). The antisera were obtained from rabbits which had been immunized by injection of 1 ml of an emulsion containing 10% mice glomerular basement membrane (GBM)-rich fractions and complete Freund’s adjuvant (CFA), intramuscularly, 4 times weekly. GBM-rich fractions were prepared according to the method of Spiro (5). The experimental
glomerulonephritis was induced by intravenous injection of NTS (0.2 ml) into mice every other day for a total of three injections.

Rabbit IgG (RGG) accelerated NTS nephritis: RGG accelerated NTS nephritis was examined by the previously described method (4). Mice were immunized by an intraperitoneal injection of 0.5 mg RGG emulsified with 0.25 ml of CFA. Five days later, NTS in a volume of 0.1 ml was injected intravenously.

Spontaneous nephritis in (NZB × NZW) F₁ mice: Fifteen week old female (NZB × NZW) F₁ mice with less than 30 mg/dl proteinuria were employed. The effect of the drug was assayed by measuring the amount of urinary protein and incidence of survivals.

Evaluation of the severity of nephritis: The urine and blood samples were collected at 1, 5, 10, 15, 20 and 25 days after the first injection of NTS. The amount of urinary protein was measured by test paper containing tetrabromophenol blue (Combi sticks II, Miles Lab.). Albumin was assayed by the bromcresol green method as described by Dounas (6). Cholesterol was measured by using acetic acid anhydride and sulfuric acid according to the method of Zurkowski (7). Blood urea nitrogen (BUN) was measured by the urease-indophenol method according to Saito et al. (8). Pathological changes in the kidney were evaluated in a semiquantitative fashion after staining with hematoxylin and eosin or periodate-Schiff according to the method of Litwin et al. (9). Examined items are swelling of glomerulus, hypercellularity, polys, crescent, lobulation, sclerosis, adhesion, interstitial infiltration and arteriolar thickening. Each of the changes were counted by scores according to 3 degrees of severity.

Statistics: Results were statistically evaluated using Student’s t-test. In histopathological studies, the statistical significance was tested by Wilcoxon’s U-test.

Results

NTS nephritis: Figure 1 shows the effect of FUT-175 on NTS nephritis in ddY mice. The increase of urinary protein and decrease of serum albumin were inhibited. The increases of cholesterol and BUN level were temporarily inhibited. In the histopathological study, hypercellularity, deposition of fibrinoid substance and the thickness of glomerular basement membrane were found in the glomelus of nephritic mice (Fig. 2). Therapy occasionally decreased the incidence of glomerular hypercellularity. However, there was no significant difference of histopathological score among the control and drug treated animals. Figure 3 shows the effect of Cy on NTS nephritis in ddY mice. The changes of biochemical parameters were clearly inhibited by administration of the drug. Histopathological changes were slightly improved in terms of hypercellularity and swelling of the glomelus (not significant at P<0.05). Figure 4 shows the effect of FUT-175 on NTS nephritis in A/He mice which are a complement-deficient strain. The suppression of the changes in biochemical parameters was much less than that in the
case of ddY mice. The histopathological changes in A/He mice were slight when compared with those in ddY mice. FUT-175 did not affect the increase of histopathological score in this strain.

Fig. 2. Histopathological picture of the kidney from a mouse with nephrotoxic serum nephritis. The mouse was sacrificed 25 days after the NTS injection (PAS-stain, ×200). Deposition of fibrinoid substance and thickness of glomerular basement membrane were found.

Fig. 3. Effect of cyclophosphamide on nephrotoxic serum nephritis in ddY mice. Drug was administered p.o. for 25 days after the first injection of NTS. Each point represents the mean of 4 to 10 animals. The standard error is not shown for clarity, but it is less than 26.5% in all points. *P<0.05, significantly different from the control. ○: Control, ●: 5 mg/kg, △: 20 mg/kg.

Fig. 4. Effect of FUT-175 on nephrotoxic serum nephritis in A/He mice. Drug was administered p.o. for 25 days after the first injection of NTS. Each point represents the mean of 4 to 8 animals. The standard error is not shown for clarity, but it is less than 38.2% in all points. *P<0.05, significantly different from the control. ○: Control, ●: 100 mg/kg.

RGG accelerated NTS nephritis: Figure 5 shows the effect of FUT-175 on RGG accelerated NTS nephritis in ddY mice. The changes of biochemical parameters, especially proteinuria and serum albumin, were inhibited by FUT-175. In histopathological studies, the main changes of this nephritis were crescent formation and deposition of fibrin or fibrinoid substance in the capsule (Fig. 6). The elevation of histopathological score in the control group was not affected by FUT-175. Figure 7 shows the effect of Cy on RGG accelerated NTS nephritis in ddY mice. The changes of urinary and serological parameters were clearly inhibited. The inhibition by Cy was more effective than that by FUT-175. Histopathological changes in nephritic
mice were not significantly influenced by Cy. In (NZB×NZW) F1 mice: In (NZB×NZW) F1 mice, FUT-175 showed a remission of development of nephritis in terms of the increase of urinary protein and the incidence of surviving animals (Fig. 8).

Discussion

The present study indicates the beneficial effects of FUT-175 on experimental glomerulonephritis in mice. The therapy with FUT-175 mainly prevented the changes of proteinuria and serological parameters in all examined nephritis models. However, the histopathological changes were little affected by the drug. Similar results were obtained by Cy in nephritis models of ddY mice. At present, Cy is one of the most useful drugs for the treatment of nephritis. In contrast to its

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**Fig. 5.** Effect of FUT-175 on rabbit IgG accelerated NTS nephritis in ddY mice. Drug was administered p.o. for 25 days after the injection of NTS. Each point represents the mean of 7 to 10 animals. The standard error is not shown for clarity, but it is less than 19.8% in all points. *P<0.05 and **P<0.01, significantly different from the control. ○: Control, ●: 50 mg/kg, △: 100 mg/kg.

**Fig. 6.** Histopathological picture of the kidney from a mouse with NTS nephritis accelerated with RGG. The mouse was sacrificed 25 days after NTS injection (PAS-stain, ×200). Crescent formation, deposition of fibrin in capillary and adhesion of glomerular tuft to Bowman capsule were found.

**Fig. 7.** Effect of cyclophosphamide on rabbit IgG accelerated NTS nephritis in ddY mice. Drug was administered p.o. for 25 days after the injection of NTS. Each point represents the mean of 6 to 10 animals. Standard error is not shown for clarity, but it is less than 26.3% in all points. *P<0.05 and **P<0.01, significantly different from the control. ○: Control, ●: 5 mg/kg, △: 20 mg/kg.
clinical effectiveness, Cy did not improve histopathological changes in the employed models. These results suggest the difficulty in determining the efficacy of drugs by measuring the histopathological scores in these experimental models. It is desired to develop a new experimental model for evaluation of drug activity by means of pathological study.

With regards to the action of FUT-175, the mechanisms responsible for the beneficial effects of the drug have not been defined. At least from our present data, FUT-175 was found to be effective for the nephritis of both normal and complement-deficient mice. These data suggest the existence of multiple mechanisms in the effect of FUT-175 on nephritis. One of the possible mechanisms may be related to the anti-complement activity because there are some reports indicating the importance of complement for the onset or development of nephritis and the clear anti-complement activity of FUT-175 (10-14). However, we could not demonstrate directly the participation of anti-complement activity in the therapy for nephritis. Further investigation measuring the levels of complement in the serum or tissue is necessary.

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