Effect of Captopril Treatment on Proteinuria in NZB/NZW F₁ Hybrid Mice

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Abstract—Oral treatment of female NZB/NZW F₁ hybrid mice with captopril prevented the development of proteinuria and prolonged survival in mice demonstrating slight (trace to 1+) proteinuria. Captopril treatment also markedly reduced the incidence and magnitude of proteinuria and prevented death in mice showing significant (3+ or greater) proteinuria. Histopathological studies of the kidneys of treated mice further demonstrated improvements in the renal lesions of autoimmune mice. The mechanisms whereby captopril treatment influences the course of disease in NZB/NZW mice are not known.

Captopril, an orally active inhibitor of angiotensin-I converting enzyme, has been widely introduced as an effective anti-hypertensive drug. With accumulation of clinical cases, however, side-effects have occasionally been seen (1, 2). The side-effects already reported include proteinuria (3) and nephrotic syndrome (4), and renal biopsies on these subjects have often demonstrated changes typical of an immune-complex-induced membranous glomerulonephritis (5). In contrast to these reports, Takeda et al. (6) have indicated that proteinuria was improved during treatment with captopril in a case of renovascular hypertension associated with nephrotic syndrome.

To examine experimentally whether captopril aggravates or improves renal failure, we investigated the effect of consecutive oral administration of a high dose of captopril on the development of proteinuria and survival of the F₁ hybrid of New Zealand black and white (NZB/NZW) female mice, which are known to spontaneously develop an immune-complex-induced glomerulonephritis (7, 8).

Female NZB/NZW mice aged 4 to 6 weeks were purchased from Olac Company, Bicester, Oxon, England. These mice were obtained repeatedly in small numbers over short time intervals and housed 10 to a cage

in an animal-bearing apparatus (Anipack-L, Clea-Japan Inc., Tokyo) maintained at 22±2°C and 55±10% humidity. The animals were allowed powder feed (CA-1, Clea-Japan Inc., Tokyo) in spill-proof containers and water ad libitum. Captopril (Squibb, Princeton, NJ) was mixed with the powder feed to provide a daily dose of 15 mg/kg or 150 mg/kg and fed to the animals for 10 consecutive weeks when their urinary protein level exhibited the conditions described below. Group 1 animals (mice with slight cases of proteinuria) were begun on the captopril-containing diet or control diet on detection of trace to 1+ (30 mg/dl) urinary protein levels, with 20 mice in each group. Nearly 50% of the mice developed 1+ proteinuria at the start of the experiment. Group 2 animals (mice with significant cases of proteinuria) were begun on the captopril-containing diet or control diet on detection of urinary protein levels above 3+ (300 mg/dl), with 20 mice in each group. Nearly 40% of the mice developed 4+ (≥1,000 mg/dl) proteinuria at the start of the experiment. Since all the animals were selected using only the urinary protein level as an index, they were not strictly age-matched in each group. Thus group 1 and group 2 animals were 18–26 weeks of age and 28–36 weeks of age, respectively, at the beginning of the captopril

treatment.

Urinary protein was measured with standard dipsticks (Combitec II, Miles-Sankyo Co., Tokyo) on freshly expressed urine samples for all animals at weekly intervals. The dipsticks were assessed by observers who were not aware of which treatments the mice were subjected to. Renal tissues from surviving animals in group 2 were fixed in 10% formalin solution at the termination of captopril treatment, and 5 μ m thick paraffin sections were prepared. They were stained with hematoxylin-eosin, PAS and Azan-Mallory, and then subjected to histopathological examination under light microscopes. The slides were assessed by observers who were not familiar with the treatment regimen. Proteinuria and survival were compared statistically using a logrank- χ^2 -analysis (9).

Figure 1 (A and B) shows the effect of captopril treatment on the development of proteinuria and survival in group 1 animals. Captopril was given to mice for 10 consecutive weeks, and both proteinuria and survival

were determined for a further 10 weeks. As shown in Fig. 1A, nearly half the untreated control mice versus none of the captopril-treated mice developed 2+ or greater proteinuria by the end of the captopril treatment ($P<0.01$). More than 90% of the captopril-treated animals exhibited only trace proteinuria at this period. This protective effect of captopril was also seen when proteinuria was assessed several weeks after termination of the treatment. Thus 45% of the untreated control mice versus 8–11% of the captopril-treated mice developed 2+ or greater proteinuria at 4 weeks after the termination of treatment ($P<0.05$). Although there was no difference in survival between mice treated with 150 mg/kg captopril and the controls, the survival in mice treated with 15 mg/kg captopril was significantly ($P<0.05$) prolonged as compared with the controls at 8 weeks after the termination of treatment (Fig. 1B). These results suggest a protective effect of captopril on the development of renal failure in NZB/NZW mice showing only trace

Fig. 1. Effects of captopril treatment on the development of proteinuria and survival of NZB/NZW mice. Captopril was given to mice for 10 consecutive weeks, and both proteinuria and survival were determined for group 1 (A, B) and group 2 (C, D) animals at weekly intervals. Proteinuria: □, trace; ▨, 1+ (30 mg/dl); ▤, 2+ (100 mg/dl); ▩, 3+ (300 mg/dl); ■, 4+ ($>1,000$ mg/dl). ○—○, mice treated daily with 15 mg/kg captopril; △—△, mice treated daily with 150 mg/kg captopril; ●—●, untreated control mice.

to 1+ proteinuria.

The effect of captopril treatment on the incidence of significant (3+ or greater) proteinuria and survival in group 2 animals were then investigated. As shown in Fig. 1C, a marked decrease in the incidence of significant proteinuria was demonstrated in mice given either 15 or 150 mg/kg captopril, whereas no decrease at all was observed in untreated control mice. Thus all the untreated control mice versus 7–17% of the captopril-treated mice exhibited significant proteinuria by the end of the treatment ($P<0.01$). More than 70% of the captopril-treated animals were found to develop only trace proteinuria in this period. The difference in survival between the treated and control mice were statistically significant to the $P<0.01$ level with 15 mg/kg captopril treatment, and to the $P<0.05$ level with 150 mg/kg captopril treatment (Fig. 1D).

Histopathological findings in group 2 animals are listed in Table 1. As shown, hyalinized thickening of the capillary basement membrane and fibrinoid cap within the lumen were detected in 3 cases of the untreated control animals (75%). These serious

pathologic conditions were found in 20–45 out 100 glomeruli. Exudates deposited in Bowman's capsule accompanied by crescent formation were occasionally found. The distal convoluted tubules were mostly enlarged and the lumen contained a significant eosinophilic hyaline cast. In addition, in the interstitial tissues, some infiltrating proliferation mainly present in small lymphocytes around the interlobar arteries were recognized. A decrease of red blood cells in glomerular capillaries was also a frequent finding. These renal lesions similar to so-called systemic lupus erythematosus (SLE) closely resembled examples shown in the literature (10). Although the number of cases examined histologically was small, renal lesions seemed not to present any problem in the control animals. On the other hand, as to the captopril-treated animals, the histopathological findings corresponding to the renal lesions of each untreated control animal were observed in one of 7 cases of 15 mg/kg captopril treated animals and one of 4 cases of 150 mg/kg captopril treated animals. Namely, in 9 of 11 cases of all the captopril-treated animals (about 80%), the

Table 1. Histopathological findings of renal tissue in captopril-treated NZB/NZW mice

Captopril dose (mg/kg)	Animal No.	Thickening of the glomerulus capillary basement membrane*	Protein-like substances in Bowman's space	Crescent formation in Bowman's capsule	Hyaline cast in the renal tubules	Enlarge-ment of renal tubules	Infiltrating proliferation of lymphocytes around the interlobar arteries
Control	1	+ (4)**	– (0)**	– (9)**	+	–	+
	2	+++ (38)	+	+	+	–	++
	14	++ (19)	++ (20)	+	++	+	++
	15	+++ (45)	+	+	+++	++	+
15	3	– (0)	– (0)	– (0)	–	–	++
	4	– (0)	– (0)	– (0)	–	–	+
	5	+	– (0)	– (0)	+	+	++
	6	– (0)	– (0)	– (0)	+	+	++
	7	– (0)	– (0)	– (0)	–	–	++
	8	– (0)	– (0)	– (0)	+	–	++
	9	++ (25)	+	– (0)	+	+	++
150	10	– (0)	– (0)	– (0)	+	–	+
	11	++ (20)	+	+	+++	+++	+
	12	– (0)	– (0)	– (0)	+	–	++
	13	– (0)	– (0)	– (0)	++	++	++

* accompanied by fibrinoid cap in the lumen. ** the figure in the parenthesis indicates the number of lesions of 100 each of glomeruli or Bowman's capsules. –=Negative result, +=Positive result (slight), ++=Positive result (moderate), +++=Positive result (severe).

glomerular lesions could be scarcely detected under the present examination. The above results indicate that treatment of NZB/NZW mice with captopril in fact decreases the incidence of renal failure in these animals and during the treatment, prevented death.

Since the suppressive effect of captopril on the incidence of significant proteinuria in group 2 animals was noted quite soon after administration, it seems likely that captopril influences the permeability of the glomerular capillary wall. In this regard, it should be noted that captopril is a specific inhibitor of angiotensin-I converting enzyme, and that the administration of renin or angiotensin II has been known to produce proteinuria through the enhancement of glomerular filtration of protein due to its pressure effect (11) or a change of glomerular permeability (12) and decreased tubular protein reabsorption. It is, therefore, possible that the dramatic decrease of proteinuria in these animals resulted from the inhibition of the proteinuria-inducing action of angiotensin II by captopril. However, further studies are needed to confirm this assumption.

Although it is not known why captopril treatment protects and improves NZB/NZW mice against glomerulonephritis and death, the present investigation adds an important piece of information on the renal effect of captopril in patients with autoimmune disorders.

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