Reduction of the Mortality Rate by Imidapril in a Small Coronary Artery Disease Model, (NZW × BXSB)F1 Male Mice

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ABSTRACT—For this study, we used (NZW × BXSB)F1 male mice as a model of myocardial infarction. The animals were kept on water containing imidapril or enalapril at 60 mg/kg/day from 10 to 27 weeks of age. Imidapril and enalapril significantly reduced the blood pressure. Imidapril reduced the mortality rate more significantly than enalapril did. In the second experiment where imidapril, enalapril and captopril were administered to the mice at 5 mg/kg/day, p.o., both imidapril and captopril significantly reduced the mortality, but enalapril did not. Blood pressure was slightly reduced by these ACE inhibitors. These data suggest that imidapril and captopril are efficacious for the treatment of myocardial infarction and blood pressure reduction hardly contributes to its mechanism of action.

Keywords: Imidapril, (NZW × BXSB)F1 male mice, Myocardial infarction

Hypertension has been a leading risk factor for cardiovascular diseases (1). In hypertensive subjects treated with antihypertensive agents, the risk of heart disease has been reported to decline compared with that in untreated hypertensive subjects (2). Mechanisms for the action of antihypertensive agents on the heart disease have been suggested to be a direct action on the myocardium and/or the reduction of ventricular afterload due to the arterial vasodilation. Many investigators have been trying to elucidate the detailed mechanisms using experimental models. Although ischemic heart disease in experimental animals induced by coronary occlusion has been often reported (3), there hardly exist any animal models for spontaneous and chronic heart failure in humans.

High incidences of small coronary artery disease (SCAD) and myocardial infarction (MI) in addition to acute lupus has been found in (NZW × BXSB)F1 [(W × B)F1] male mice (4, 5). Recently, it has been revealed that their mortality rate was 0% at 12 weeks, 9% at 16 weeks and 37% at 24 weeks. The incidence of MI in the surviving mice was 0% at 12 weeks, 22% at 16 weeks and 53% at 24 weeks (6). Their mean age at death was 24.7 weeks, and 65% of them died by the age of 51 weeks; the incidence of MI was 96% in the dead mice and 57% in the surviving ones at the age of 51 weeks (7). Therefore, (W × B)F1 male mice are thought to be a good experimental model for chronic and spontaneous SCAD.

In 1992, it was reported that chronic treatment of (W × B)F1 with nifedipine reduced their mortality and the incidence of occlusive thrombi in the small coronary arteries (8). The authors suggested that nifedipine has a preventive effect against SCAD through hypotension and its calcium antagonistic action. Angiotensin converting enzyme (ACE) inhibitors have not been investigated yet.

This study was performed to investigate the effectiveness of imidapril, a novel ACE inhibitor (9), on myocardial ischemia using (W × B)F1 male mice.

(W × B)F1 male mice were prepared by mating NZW: NCrj females (Charles River Japan, Inc., Kanagawa) with BXSB males (Jackson Laboratory, Bar Harbor, ME, USA). The (W × B)F1 male mice were weaned at 3-4 weeks of age and raised until they were 10-week-old. All mice were housed in air conditioned rooms at a temperature of 23±1℃ with a humidity of 55±5% on a 12-hr light-dark cycle, and they were provided with tap water and standard laboratory diet (CRF-1; Oriental Yeast Industry Co., Ltd., Chiba) ad libitum. Imidapril hydrochloride or enalapril maleate, both synthesized at the Research Laboratory of Applied Biochemistry, Tanabe Seiyaku Co., Ltd., was dissolved in distilled water to a concentration of 0.03%, respectively. At the age of 10

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weeks, 61 (W × B)F1 male mice were divided into the following 3 groups: control group (n=27), imidapril (0.03%) group (n=18) and enalapril (0.03%) group (n=16). At this time, the tap water of each group was replaced by distilled water, 0.03% imidapril hydrochloride or enalapril maleate solution. The daily dose calculated from the drinking fluid intake was about 60 mg/kg body weight throughout the medication period. Systolic blood pressure (SBP) and heart rate (HR) were measured by the tail-cuff method (more than 6 mice in each group; blood pressure monitor MK-1000; Muromachi Kikai Co., Ltd., Tokyo) at intervals of 5 weeks. The mortality of the mice was observed every day until the animals became 27-week-old. The survival rate, estimated by the Kaplan-Meier method, was statistically analyzed by the log rank test. Other values were analyzed by Scheffe's multiple comparison. A value of $P<0.05$ was considered to be significantly different.

Figure 1 shows the survival rate and mean values of SBP in each group of this experiment. Slight elevation of blood pressure was observed in the control. Blood pressure in the imidapril and enalapril groups was significantly decreased and followed by slight age-related elevation, although the pressure remained at a lower level than that of the control group. Mean values of HR in the ACE inhibitor groups were slightly lower than that in the control group (data not shown). There were statistical differences among the three survival rates ($\chi^2=9.56$, df=2: $P=0.0084$). The survival rate in the control group markedly decreased after the age of 15 weeks, and it was 37% (10/27) when the mice were 27-week-old. In the imidapril group, 78% (14/18) were still alive at 27 weeks of age, and the survival rate throughout the medication period was significantly higher than that in the control group ($P=0.005$). The survival rate in the enalapril group during the medication period was slightly higher than that in the control, although this was not statistically significant ($P=0.0510$).

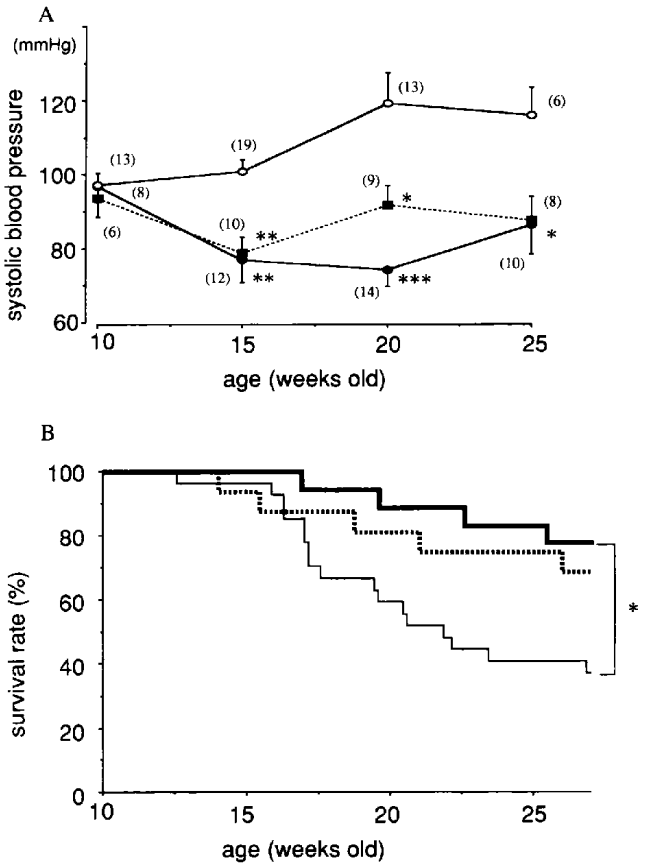


Fig. 1. Effects of imidapril and enalapril on blood pressure and life span in (NZW × BXSB)F1 male mice. Mice were maintained on drinking solution including 0.03% imidapril hydrocholride (● in graph A; —— in graph B, n=18) or enalapril maleate (■ in graph A; ····· in graph B, n=16) from the age of 10 weeks. Values of systolic blood pressure are given as the mean±S.E.M. The numbers in parentheses are the numbers of animals used for measuring blood pressure. Values of systolic blood pressure and the survival rate were statistically analyzed by Scheffe's multiple comparison and log rank test, respectively. *: $P<0.05$, **: $P<0.01$, ***: $P<0.001$ vs. control (○ in graph A; —— in graph B, n=27).

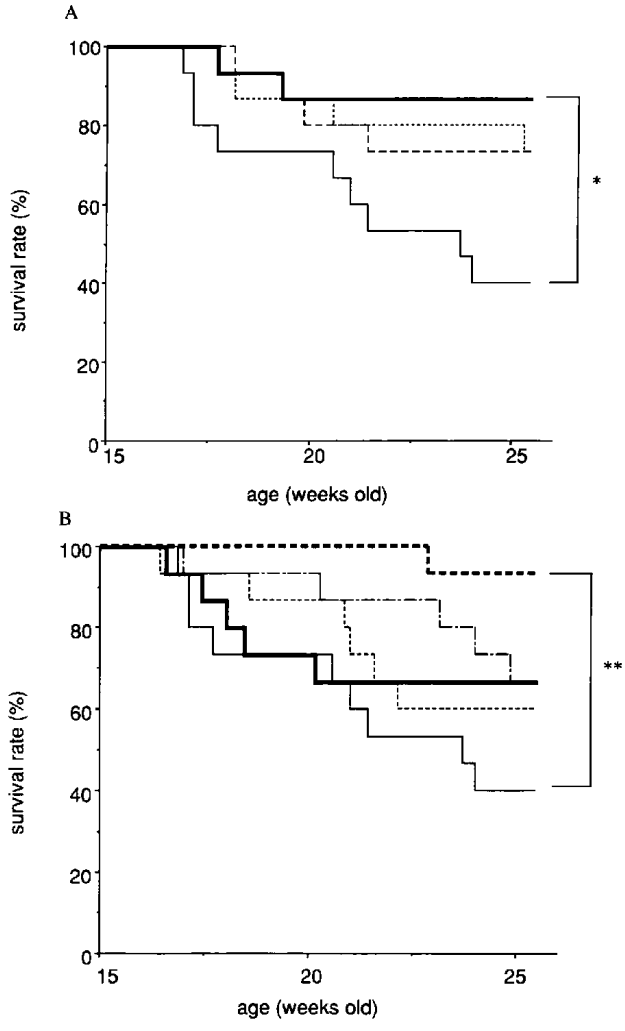
To determine the minimum effective dose of imidapril, another 128 (W × B)F1 male mice were prepared and kept under the same conditions and used for the dose-dependency study. Because 8 of the mice showed a sharp increase in body weight until they were 15-week-old, they were omitted from the experimental group. The remaining 120 mice were divided into the following 8 groups (n=15) at the age of 15 weeks on the bases of body weight and SBP: control; imidapril: 0.5, 2 and 5 mg/kg/day; enalapril: 0.5, 2 and 5 mg/kg/day; and captopril, 5 mg/kg/day. These drugs were dissolved in distilled water and administered daily to the mice by gavage at a volume of 10 ml/kg for 10 weeks. Captopril was obtained by extraction from tablets of Captoril™ (Sankyo Co., Ltd., Tokyo) in the Research Laboratory of Applied Biochemistry, Tanabe Seiyaku Co., Ltd. Blood pressure and HR were measured as described above. Body weight was measured once a week. As soon as the mice were found to be dead, they were autopsied.

Table 1 shows mean values of SBP, HR and double product (SBP × HR), and Fig. 2 shows the survival rate in the dose-dependency study. There were statistically significant differences among the eight survival rates ($\chi^2=14.10$, df=7: $P=0.0495$). In the control group, the survival rate decreased, and the mean values of SBP, HR and double product slightly increased in an age related manner. These values were almost equal to those of the age-matched mice in the control group in the first experiment. In all of the ACE-inhibitor-treated groups, the mean values of SBP, HR and double product were slight-

Table 1. Effects of ACE inhibitors on blood pressure, heart rate and double product in (NZW × BXSB)F1 male mice

Drug	Dose (mg/kg)	Systolic blood pressure (SBP: mmHg)			Heart rate (HR: beats/min)			SBP × HR (mmHg × beats/min)		
		15	20	25	15	20	25	15	20	25 (weeks old)
Vehicle	—	107.8± 6.6	118.8± 9.8	119.5±12.5	595.2±32.0	543.4±41.5	626.3±43.9	64083.5±4723.4	66381.9±9912.3	77126.4±13356.2
Imidapril	0.5	108.1± 3.6	113.6± 5.3	118.1± 6.5	552.1±26.9	517.6±31.5	504.6±27.7	59267.4±2866.8	59512.1±5717.8	59059.8± 3790.5
	2.0	106.5± 4.1	111.1± 4.7	113.9± 6.3	549.5±24.9	475.4±34.8	500.0±32.0	58468.4±3358.3	51631.8±2869.6	57735.2± 5789.5
	5.0	107.6± 3.6	104.8± 5.4	103.5± 6.5	547.7±24.7	491.5±24.1	486.7±19.0	59206.9±3674.9	51168.5±3230.7	51131.0± 4806.4
Enalapril	0.5	107.0± 3.6	124.1± 9.0	108.3± 9.1	581.0±28.6	481.9±30.1	498.2±30.2	62293.2±3771.0	61056.3±7134.9	55705.7± 7915.8
	2.0	107.8± 2.9	118.9± 7.4	112.6± 6.7	547.0±30.0	461.7±23.9	458.8±15.7	59133.8±4111.6	55432.4±4845.2	51805.6± 3946.6
	5.0	106.7± 4.8	101.1± 5.7	106.0± 4.2	554.1±21.5	518.1±22.2	473.6±36.2	59056.2±3609.9	53071.7±4953.1	50791.1± 5296.8
Captopril	5.0	107.1± 4.0	110.1± 7.5	111.7± 7.3	596.2±24.1	511.1±22.3	537.6±26.8	64356.1±4430.9	55812.9±3913.1	60316.3± 5316.9

Drugs were administered orally once a day from 15 to 26 weeks of age. Values are the mean±S.E.M. (n=6−14). They were statistically analyzed by Scheffe's multiple comparison.



ly lower compared with those of the control group, but the differences did not reach statistical significance. At the end of the experimental period, the survival rate was 40% (6/15), 73% (11/15), 73% (11/15) and 87% (13/15) in the control and imidapril: 0.5, 2 and 5 mg/kg/day groups, respectively. In the enalapril: 0.5, 2 and 5 mg/kg/day and captopril groups, the survival rate at the end point was 67% (10/15), 60% (9/15), 67% (10/15) and 93% (14/15), respectively. Imidapril and captopril, both at 5 mg/kg/day, significantly reduced the mortality compared with the control (P=0.0104 and 0.0017, respectively). The mortality in each of the enalapril groups was reduced. However, it was not statistically different from that in the control group.

In the dose-dependency study, 9 mice died during the experimental period in the control group. Seven of them showed a sharp increase followed by decrease in body weight; thereafter, they died. In the autopsy, atriomegaly, granulations in the liver, congestive edema in the lung, accumulation of plueural effusion, and subcutaneous edema, which are typical evidence of ischemic heart desease, were observed in 5 of them. Some dead mice in the ACE-inhibitor-treated groups showed the same symptoms. The numbers of mice that showed these findings against ones that died during the experiment period were as follows: 4/4, 4/4 and 2/2 in the groups treated with imidapril: 0.5,

Fig. 2. Effects of ACE inhibitors on life span in (NZW × BXSB)F1 male mice. Drugs were administered orally once a day from 15 to 26 weeks of age. The survival rate was statistically analyzed by the log rank test. *: P<0.05, **: P<0.01 vs. control, n=15. ——: control; ·····: imidapril, 0.5 mg/kg; - - - -: imidapril, 2.0 mg/kg; ——: imidapril, 5.0 mg/kg in graph A. ——: control; - - - -: enalapril, 0.5 mg/kg; ·····: enalapril, 2.0 mg/kg; ——: enalapril, 5.0 mg/kg; - - - -: captopril, 5.0 mg/kg in graph B.

2, 5 mg/kg/day; 2/5, 4/6 and 4/5 in the groups treated with enalapril: 0.5, 2, 5 mg/kg/day; and 1/1 in the captopril-treated group. The 8 mice that were omitted from the experiment showed the same findings by autopsy.

This study revealed that imidapril and captopril are efficacious in the spontaneous MI model mice at more than 5 mg/kg/day, p.o., but the same dose of enalapril is not. The prolonging effect on the life-span may be due to the preventative effect against MI, although detailed investigations on the mechanisms are necessary. There have been very few papers reporting that ACE inhibitors were efficient against spontaneous heart failure in experimental animals other than cardiomyopathic Syrian hamsters (10). The main cause of death of the cardiomyopathic hamster is heart disease due to cardiomyopathy, while that of the (W × B)F1 mouse is heart disease due to ischemia (7). The incidence of MI in the surviving mice was reported to be 22% at 16 weeks (6). One mouse in the control and one in the enalapril group, died between 10 and 15 weeks of age in the first study. The 8 mice that were omitted from the dose-dependency study showed systemic edema by 15-week-old. Judging from these data, some mice among the 120 in the dose-dependency study had MI at 15 weeks of age. ACE inhibitors may partly delay the progress of heart disease in this model. Although other investigators did not find typical symptoms of heart failure in (W × B)F1 mice, we found evidence in the present study that some mice may have possibly developed heart failure.

Because the time courses of mortality and change of SBP in the controls of our two present experiments were quite similar and the survival rate in the control in the present study was quite consistent with those reported elsewhere (6, 7), this MI model is considered to be easily reproducible by only mating.

A general pharmacological study showed that the treatment with imidapril or enalapril at 100 mg/kg, p.o. does not affect the heart rate in cats (11). Although there were no statistical differences, the mean values of HR and double product in the ACE-inhibitor-treated groups were lower than those in the control, which increased age-relatedly in this study. It was suggested that the reduction of before and after-load, increase of the coronary flow, and reduction of contractility of the heart, which are well known effects of ACE inhibitors, brought about these changes in (W × B)F1 males in this experiment.

The antihypertensive effect of imidapril was known to be as potent as that of enalapril and 5 times more potent than that of captopril in some experimental hypertensive animal models (9). However, the effective imidapril dose for increasing the life-span in (W × B)F1 males was 5 mg/kg/day, p.o., which was almost equal to that of captopril. Imidapril and captopril significantly reduced the mortality without showing a marked hypotensive effect. Enalapril, which reduced blood pressure to the same level as imidapril, did not show the prolonging effect on life-span. It is suggested that the reduction of blood pressure contributes very little to this effect. Imidapril probably exerts its protective effect on the cardiovascular system through other mechanisms including the hypotensive effect.

The relationship between cardiomyopathy and the renin-angiotensin (RA) system has been reported (12). It is also reported that ACE inhibitors bring a better prognosis to patients suffering from heart failure than other antihypertensive drugs do (13). Several studies have suggested that the RA system is activated after MI and the extent of activation is related to the degree of left ventricular dysfunction (14). The duration of the ACE inhibiting effect of imidapril in hearts was known to be much stronger and longer lasting than that of enalapril after the same dose oral administration in rats (unpublished obsevation, Y. Hashimoto et al.). The data may explain why imidapril was more effective than enalapril in this experiment. There is a discrepancy that captopril, whose ACE inhibiting effect is one sixth that of imidapril (15), showed the same extent of effectiveness. Because of the SH-group in the molecule, captopril is known to have a radical scavenging effect. It is better for us to understand that captopril has other mechanisms besides ACE inhibition.

In conclusion, imidapril reduced the mortality in this myocardial ischemia murine model. Reduction of blood pressure is not necessary for this effect. Further studies are necessary to clarify the mechanism of its action.

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