Cardioprotective Effects of an Angiotensin-Converting-Enzyme Inhibitor, Imidapril, and Ca^{2+} Channel Antagonist, Amlodipine, in Spontaneously Hypertensive Rats at Established Stage of Hypertension

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ABSTRACT—The present study was performed to compare cardioprotective effects of an angiotensin-converting-enzyme inhibitor, imidapril, and of a Ca^{2+} channel antagonist, amlodipine, against the cardiac hypertrophy in male spontaneously hypertensive rats (SHRs) at the established stage of hypertension. Fifteen-week-old SHRs were given imidapril (2 and 5 mg/kg/day) or amlodipine (10 mg/kg/day) by gavage for 8 weeks. Three hours after the 1st treatment, imidapril moderately reduced blood pressure without changing heart rate, while amlodipine caused a marked reduction in blood pressure accompanied by transient tachycardia. At the end of the treatments, ventricular weight in the imidapril-treated groups was markedly lower, but that in the amlodipine-treated group was only slightly lower than that in the vehicle-treated group. Myocardial collagen content in the imidapril-treated group tended to be decreased, and significant reduction was observed in the low-dose group. In another experiment, the isolated heart of the imidapril-treated animals demonstrated better cardiac compliance than that in the vehicle-treated animals. In contrast, amlodipine failed to improve cardiac function. The present results suggest that imidapril possesses advantageous effects to prevent cardiac hypertrophy and deteriorated cardiac function in SHRs of established stage of hypertension as compared with amlodipine.

Keywords: Imidapril, Amlodipine, Spontaneously hypertensive rat, Cardiac hypertrophy, Cardiac function

Although the increased thickness of the ventricular wall is a consequence of adaptation in the initial stage of hypertension (1), cardiac hypertrophy is a major predisposition in congestive heart failure (2) and causes a critical outcome in the senescent heart, such as diastolic failure and deteriorated compliance in ventricular diastole (3), arrhythmia (4), and insufficient coronary blood flow (5). Numerous antihypertensive drugs have been used to control blood pressure (BP), aiming to suppress or avoid cardiac hypertrophy. Among several series of antihypertensive drugs including diuretics, beta-adrenergic blockers, Ca^{2+} channel antagonists and angiotensin-converting-enzyme inhibitors (ACEIs), ACEIs have been regarded as conspicuous antihypertrophic agents in the clinical stage (6). In in vivo and in vitro experiments, direct stimulation by angiotensin II has been reported to produce myocyte hypertrophy, cardiac hyperplasia and collagen synthesis (7–9).

Imidapril (TA-6366) is a prodrug ACEI with long-lasting effect whose pharmacological profiles have already been examined in detail (10–14). It has been reported that imidaprilat, the active metabolite of imidapril, is approximately 3 times as potent as enalaprilat in vitro (13). Moreover, also in vivo experiments using 2-kidney, 1-clip Goldblatt hypertensive rats and spontaneously hypertensive rats (SHRs), imidapril has been reported to cause a similar or potent long-lasting hypotensive effect as compared with enalapril or captopril (10). Concerning the effect of imidapril to prevent the development of cardiac hypertrophy, Kubo et al. (11) reported its favorable effects in 4-week-old SHRs. However, there are no reports about the reduction of the hypertrophy or improvement of cardiac function in SHRs of the established stage of hypertension.

On the other hand, Ca^{2+}-channel antagonistic dihydropyridine (DHP) analogues have been widely used for the treatment of hypertensive patients. These compounds reduce the burden in the heart of the patients mainly

through a decrease in peripheral vascular resistance (15, 16). Among the compounds, amlodipine is a representative one with gentle and long-lasting activity (17). However, the antihypertrophic activity of the agent in the heart is still controversial (18, 19).

In the present study we aimed to clarify the effect of imidapril on the heart with developed hypertrophy in SHRs of the established stage of hypertension. For this purpose, 15-week-old SHRs were used because the significant increase in the thickness of heart wall compared to that of Wister Kyoto rats (WKY) becomes distinct at around 13 weeks of age (20). The animals were given imidapril orally for 8 weeks. The BP was monitored and the tissue weight and the collagen content of the ventricle were measured. Additionally, the effects of amlodipine were studied following the same protocol as that for imidapril.

MATERIALS AND METHODS

All the experiments were carried out with the approval of the Committee for Ethical Use of Experimental Animals in Hatano Research Institute.

Measurement of blood pressure, heart rate, ventricular weight and myocardial collagen content

Forty 15-week-old SHRs (Charles River Japan, Inc., Tokyo) were divided into 4 groups of 10 animals of each, and they were given vehicle, imidapril (2 or 5 mg/kg) or amlodipine (10 mg/kg) daily by gavage for 8 weeks. To clarify the relationship between the hypotensive effect and antihypertrophic effect, doses of the drugs used in the present study were chosen according to the previous reports (10, 18). Imidapril at 5 mg/kg or amlodipine at 10 mg/kg were expected to cause marked reductions of BP, and 2 mg/kg of imidapril was expected to cause a minimal effect or smaller reduction of BP than 5 mg/kg of the drug. Systolic BP and heart rate (HR) were measured with tail plethysmography (MK-1030; Muromachi, Tokyo) on the first day of the treatment and thereafter every 2 weeks, before and 3 hr after the administration. After the 8-week treatment, the animals were anesthetized with 60 mg/kg of intraperitoneal pentobarbital sodium and sacrificed by exsanguination. The heart was removed via a midline thoracotomy and rinsed in 0.9% saline. After removing the atrium, the ventricle was divided into the left (LV with the septum) and the right ventricle (RV). The wet weight of each was measured and then each was separately homogenized in 9 vol. of 0.9% saline and stored at −135℃ until subsequent assay. An aliquot of homogenate (0.1−0.25 ml) was hydrolyzed by HCl at the final concentration of 6 N at 110℃ for 21 hr. After neutralizing by KOH, the content of 4-hydroxyproline was determined using *trans*-4-hydroxy-L-proline (0.5−6 μg/ml; Sigma, St. Louis, MO, USA) as a standard. The sample or standard was saturated by KCl and then incubated with 0.25 ml of 10% L-alanine (pH 8.7, Sigma) and 0.5 ml of 1 M borate-KOH buffer (pH 8.7) containing 3 M KCl for 30 min at room temperature. The incubation was continued for 25 min at room temperature after adding 0.5 ml of 0.2 M chloramine T (Wako, Tokyo) dissolved in 2-methoxyethanol. The reaction mixture was added with 1.5 ml of 3.6 M sodium thiosulfate and then was shaken for 3 min with 2.5 ml of toluene. The mixture was centrifuged at $380 \times g$ for 5 min, and the aqueous layer obtained was heated in boiling water. Then the mixture was cooled in water and shaken with 2.5 ml of toluene again. After centrifugation at $380 \times g$ for 5 min, 1.25 ml of the toluene layer was transferred to another tube and was incubated for 30 min at room temperature with 0.5 ml of 1.88 M *p*-dimethylaminobenzaldehyde dissolved in 1.2 M H_2SO_4/ethanol. Its absorbance at 560 nm was measured by a spectrophotometer (UVIDEC-610C; Nihon Bunko, Tokyo). The content of collagen in each sample was estimated by multiplying the concentration of 4-hydroxyproline by 8.2 (21).

Evaluation of cardiac function by working heart method

Forty other 15-week-old SHRs were treated with the vehicle, imidapril or amlodipine in the same-manner as experiment 1, except for the measurement of BP and HR (only the pre-dosing levels were measured to confirm the effects of repeated administration).

After the 8-week administration, the cardiac function was evaluated in the working heart preparation according to the method of Neely et al. (22). Briefly, the animal was anesthetized with urethane (1 g/kg, i.p.; Aldrich Japan, Inc., Tokyo), and heparin sodium was injected (500 UI, i.v.; Sigma). Then the animal was sacrificed by exsanguination, and the heart was quickly removed via a midline thoracotomy. A stainless steel cannula was inserted into the aorta, and the heart was then perfused retrogradely with Krebs-Henseleit (KH) solution (warmed to 37℃ and equilibrated with 95% O_2 − 5% CO_2 gas) at a perfusion pressure of 68 cmH_2O (approximately 50 mmHg). The composition of KH solution was: 117.54 mM NaCl, 5.37 mM KCl, 2.52 mM $CaCl_2$, 25.00 mM $NaHCO_3$, 1.17 mM $NaHPO_4$, 1.16 mM $MgSO_4$ and 11.10 mM glucose. A stainless steel cathether, which was connected to a reservoir containing KH solution, was inserted into the left atrium, and the heart was perfused with its own output under the condition of 5 cmH_2O preload and 68 cmH_2O afterload. Aortic pressure (AoP) was measured with pressure transducers (P23XL; Spectramed, Oxnard, CA, USA) connected to an air chamber (an air room for aortic compliance) and a polygraph (Model 363; NEC-San ei, Tokyo). HR was measured with a tachometer (T-149;

Data Graph, Tokyo) triggered by AoP pulses. Aortic flow through the aortic catheter and coronary flow through the pulmonary arterial catheter were measured with electromagnetic flowmeters (MV-470; Nihon Kohden, Tokyo). Cardiac output (CO) was calculated by adding coronary flow (CoF) to the aortic flow. Consequently, CO and CoF were divided by total ventricular (LV and RV) weight, and they were represented as CO/VW and CoF/VW, respectively. In 4–6 animals of each group, a polyethylene tube tipped with an injection needle (22G) connected to a pressure transducer (P23XL) was inserted into the left ventricle via the apex to measure peak left ventricular pressure (PLVP) and end-diastolic pressure (LVEDP). After a 10-min stabilization, preload (the surface level of KH solution in the reservoir) was elevated by 5-cm steps to 20 cmH_2O and changes in the parameters were recorded (Recti-Horiz 8K, NEC-San ei).

Drugs

Amlodipine was chemically extracted from tablets for clinical use (Norvasc; Pfizer, Tokyo), and imidapril was synthesized by Tanabe Seiyaku. Amlodipine and imidapril were dissolved in a mixture of the following solutions: ethanol, 15; polyethylene glycol 200, 15; water, 70, in volume percentage. Chemicals used for the vehicle and other reagents used in the present study were of the finest grade commercially available.

Statistical analyses

The results are represented as means±S.E. in the present study. ANOVA accompanied by Fisher's PLSD was employed for group comparison. The difference in the mean values was considered significant at $P<0.05$.

RESULTS

Blood pressure and heart rate

Mean values of systolic BP measured by tail plethysmography were 183±3, 185±3 and 188±4, and 198±3 mmHg in the groups treated with vehicle, 2 and 5 mg/kg/day of imidapril, and 10 mg/kg of amlodipine, respectively. Imidapril at 2 and 5 mg/kg/day caused a mild, dose-dependent reduction in BP without any marked changes in HR, while 10 mg/kg/day of amlodipine caused a marked reduction of BP that was accompanied by transient tachycardia (+10.7±3.1% of preadministration level, Fig. 1). In contrast, in the group treated with only the vehicle for 8 weeks, BP tended to increase. In the imidapril- and amlodipine-treated groups, the spontaneous elevation in BP was completely suppressed (Fig. 2). Moreover, in the amlodipine-treated group, the pre-dosing level of HR was significantly lower at the 2nd, 4th, 6th and 8th week ($P<0.01$ or $P<0.001$ for each) than that before starting the administration.

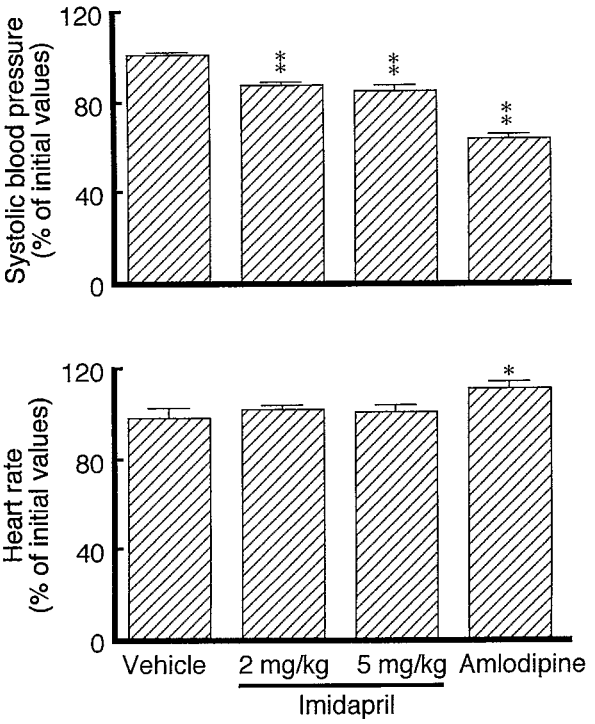

Fig. 1. Effects of single oral administration of vehicle, 2 and 5 mg/kg/day imidapril, and 10 mg/kg/day amlodipine on systolic blood pressure (upper panel) and heart rate (lower panel) in SHRs (n=10 for each group) measured on the 1st day of the treatments. The measurement was carried out before and at approximately 3 hr after the 1st administration. *$P<0.05$, **$P<0.01$ vs vehicle group (by Fisher's PLSD).

Ventricular weight and myocardial collagen content

Both the absolute and relative LV weight and RV weight were reduced dose-dependently by imidapril. They were significantly lower as compared with those of the vehicle-treated group (Table 1). Total ventricular weight (TVW) in the amlodipine-treated group was slightly but significantly lower than that in the vehicle-treated group, whereas the reduction in each portion (LV and RV) was insignificant.

In the imidapril-treated groups, myocardial collagen contents tended to be lower than that in the vehicle-treated group, although a significant decrease was observed only in the 2 mg/kg/day-treated group. That in the amlodipine-treated group did not change (Table 2). Additionally, myocardial collagen concentration was not affected clearly by imidapril (Table 2).

Cardiac function

TVW in the groups given vehicle, 2 and 5 mg/kg of imidapril, and amlodipine were measured after examining the cardiac function; and the values were 2.03±0.04,

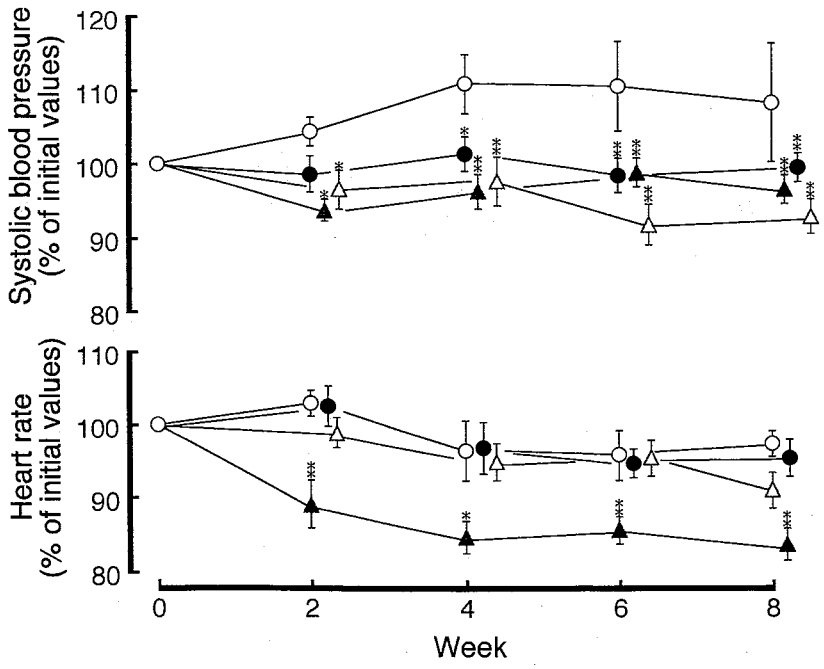

Fig. 2. Effects of repeated oral administration of vehicle (○), 2 and 5 mg/kg/day imidapril (● and △, respectively) and 10 mg/kg/day amlodipine (▲) for 8 weeks on systolic blood pressure (upper panel) and heart rate (lower panel) in SHRs (n=10 for each group). The measurement was carried out before administration. *P<0.05, **P<0.01 vs vehicle group (by Fisher's PLSD).

Table 1. Body weight and myocardial ventricular weight in 23-week-old SHRs treated with imidapril or amlodipine for 8 weeks by gavage

Drug	BW (g)	Absolute ventricular weight ($\times 10^{-2}$ g)			Relative ventricular weight to BW ($\times 10^{-4}$)		
		LVW	RVW	TVW	LVW	RVW	TVW
Vehicle	374.5±5.2	91.6±1.2	24.8±1.7	116.4±1.8	24.5±0.5	6.6±0.4	31.1±0.4
Imidapril, 2 mg/kg	361.9±4.8$^{\#}$	83.5±1.2**	20.7±0.3**	104.2±1.2**,##	23.1±0.5**	5.7±0.1*	28.8±0.2**
Imidapril, 5 mg/kg	366.8±6.3	78.6±1.4**	20.0±0.8**	98.6±1.2**,#	21.5±0.5**	5.5±0.2**	26.9±0.5**,##
Amlodipine, 10 mg/kg	379.7±6.0	89.1±1.5	22.7±0.8	111.8±1.2*	23.5±0.2	6.0±0.2	29.5±0.2**

Values are means±S.E. of 10 animals. BW, LVW, RVW and TVW are body, left (including septum), right and total ventricular weight, respectively. *P<0.05, **P<0.01, significantly different from the values in vehicle-treated animals, and $^{\#}$P<0.05, $^{\#\#}$P<0.01, significantly different from the values in amlodpine-treated animals (by Fisher's PLSD).

Table 2. Myocardial collagen concentration and contents in 23-week-old SHRs treated with vehicle, imidapril or amlodipine for 8 weeks by gavage

Drug	Concentration (mg/g)			Contents (mg)		
	LV	RV	TV	LV	RV	TV
Vehicle	3.91±0.37	4.44±0.54	4.02±0.36	3.61±0.38	1.10±0.16	4.71±0.46
Imidapril, 2 mg/kg	3.14±0.29	3.94±0.41	3.30±0.30	2.64±0.26*	0.81±0.08	3.45±0.03*
Imidapril, 5 mg/kg	3.85±0.52	4.17±0.40	3.93±0.47	3.04±0.43	0.83±0.08	3.87±0.47
Amlodipine, 10 mg/kg	4.12±0.26	3.73±0.28	4.04±0.22	3.67±0.24	0.84±0.07	4.51±0.26

Values are mesns±S.E. of 10 animals. LV, RV and TV are left (including septum), right and total ventricle, respectively. *P<0.05, significantly different from the values in vehicle-treated animals (by Fisher's PLSD).

1.81±0.14 and 1.73±0.06, and 2.01±0.07 g, respectively. TVW in the 2 and 5 mg/kg imidapril-treated groups were significantly lower compared to that in the group treated with the vehicle or amlodipine ($P<0.01$ for each).

CO/VW in the group given imidapril at 2 mg/kg/day was significantly increased at 20 cmH_2O of preload ($P<0.05$) compared to that of the vehicle-treated group, and the group treated with 5 mg/kg/day of imidapril showed significantly increased CO/VW at all the preload levels ($P<0.01$, Fig. 3). Systolic aortic pressure (SAoP) of the group given imidapril at 5 mg/kg/day was higher than that of the vehicle-treated group and at 15 and 20 cmH_2O of preload, SAoP was significantly higher than those in the vehicle-treated group ($P<0.05$). Neither CO/VW nor SAoP was improved by amlodipine and each of them was similar to those in the vehicle-treated group (Table 3).

No notable differences in PLVP were observed in the imidapril- and amlodipine-treated groups (Table 3). Additionally, LVEDP in the imidapril-treated groups tended to be lower, and especially in the 2 mg/kg/day group at 15 cmH_2O, preload was significantly lower ($P<0.05$) than that in the vehicle-treated group. LVEDP in the amlodipine-treated group was similar to that of the vehicle-treated group.

Basal CoF at 5 cmH_2O of preload in the imidapril- and amlodipine-treated groups tended to be higher compared with that of the group given vehicle. In the 3 groups given imidapril and amlodipine, CoF increased in parallel with the elevation of preload level, but in the vehicle-treated group, an increase of CoF was observed only at 10 and 15 cmH_2O of preload levels (Table 4). However, mean values of CoF in the amlodipine-treated group tended to be higher at each preload level compared with those of the vehicle-treated group. In the group given imidapril at 2 mg/kg/day, CoF/VW at the highest level (20 cmH_2O) of preload was significantly higher than that of the vehicle-treated group ($P<0.05$). That in the group given im-

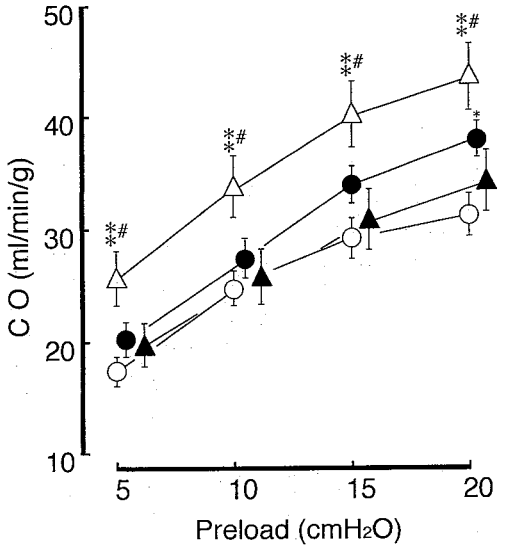

Fig. 3. Cardiac function curve indicating the influences of 8-week treatment with vehicle (○), 2 and 5 mg/kg/day imidapril (● and △, respectively) and 10 mg/kg/day amlodipine (▲) in 23-week-old SHRs (n=8−10). *P<0.05, **P<0.01 vs vehicle group, and #P<0.05 vs amlodipine group (by Fisher's PLSD).

Table 3. Cardiac function of isolated heart in SHRs treated with vehicle, imidapril and amlodipine for 8 weeks

Drug	Parameters	N	Preload (cmH_2O)			
			5	10	15	20
Vehicle	SAoP	10	76.9± 1.3	82.9± 1.9	86.7± 1.9	89.2± 2.3
	LVEDP	4	7.9± 0.6	10.4± 1.2	12.4± 1.4	14.6± 1.9
	PLVP	4	111.9± 6.8	123.8± 9.3	130.0±10.3	131.3±10.8
Imidapril, 2 mg/kg	SAoP	10	77.9± 2.2	84.9± 2.3	90.3± 2.3	93.6± 2.3
	LVEDP	5	5.4± 0.9	7.5± 0.8	8.9± 0.8*,##	11.9± 1.1
	PLVP	5	121.5±10.9	128.5±10.4	132.5± 9.3	135.0± 9.3
Imidapril, 5 mg/kg	SAoP	8	81.3± 1.9	88.6± 2.4	94.1± 2.5*	96.7± 2.3*
	LVEDP	6	6.5± 1.1	8.0± 1.1	9.5± 0.8##	11.0± 0.9#
	PLVP	6	120.2± 5.5	126.9± 4.9	132.0± 6.1	133.8± 6.9
Amlodipine, 10 mg/kg	SAoP	10	79.1± 2.3	84.4± 2.7	90.3± 2.7	92.3± 2.8
	LVEDP	5	8.2± 1.9	10.7± 1.9	13.5± 1.6	14.9± 2.0
	PLVP	5	110.0± 8.1	119.5± 7.3	122.5± 7.2	122.3± 6.7

Values are means±S.E. in mmHg. SAoP: systolic aortic pressure, LVEDP: left ventricular end-diastolic pressure, PLVP: peak left vetricular pressure. *P<0.05, significantly different from the values in the vehicle-treated group, and #P<0.05, ##P<0.01, significantly different from the values in the amlodipine-treated group (by Fisher's PLSD).

Table 4. Coronary flow and vascular resistance in isolated heart of SHRs treated with vehicle, imidapril and amlodipine for 8 weeks by gavage

Drug	N		Preload (cmH_2O)			
			5	10	15	20
Vehicle	10	CoF[a]	14.0 ±1.0	14.6 ±1.0	14.3 ±1.0	13.9 ±1.0
		CoF/VW[b]	6.95±0.59	7.25±0.59	7.10±0.59	6.91±0.59
		VR[c]	9.53±0.71	9.30±0.63	9.75±0.74	10.13±0.74
Imidapril, 2 mg/kg	10	CoF	14.7 ±0.9	15.3 ±0.6	15.7 ±0.8	15.9 ±0.7
		CoF/VW	8.20±0.61	8.53±0.48	8.77±0.57	8.87±0.54*
		VR	8.00±0.55	7.85±0.40	7.94±0.50*	7.93±0.46*
Imidapril, 5 mg/kg	8	CoF	16.4 ±1.3	17.5 ±1.2	17.9 ±1.2 *	18.0 ±1.0 **
		CoF/VW	9.62±0.93	10.25±0.81**,##	10.49±0.85**,##	10.58±0.83**,##
		VR	7.14±0.73	6.78±0.59**,#	6.73±0.56**,#	6.73±0.51**,##
Amlodipine, 10 mg/kg	10	CoF	15.1 ±1.2	15.5 ±1.2	15.6 ±1.2	15.3 ±1.3
		CoF/VW	7.55±0.63	7.73±0.58	7.80±1.64	7.67±0.68
		VR	8.93±0.78	8.80±0.65	9.00±0.69	9.37±0.81

Values are means±S.E. in [a]ml/min, [b]ml/min/g, and [c]mmHg/ml/g/min. *P<0.05, **P<0.01, significantly different from the values in the vehicle-treated group, and #P<0.05, ##P<0.01, significantly different from the values in the amlodipine-treated group (by Fisher's PLSD).

idapril at 5 mg/kg/day was significantly higher at all preload levels compared to those in the vehicle or the amlodipine-treated group. Coronary vascular resistance that was obtained by dividing the mean aortic pressure by CoF/VW in the imidapril-treated groups significantly decreased as compared with those in the vehicle- or amlodipine-treated group.

DISCUSSION

Kubo et al. reported that repeated oral administration of imidapril to 4-week-old SHRs (prehypertensive stage) for 10 weeks prevented the development of hypertrophy of the heart (10). In the present study, we confirmed the regression by imidapril of the cardiac hypertrophy also in 15-week-old SHRs at the hypertensive stage. The oral administration of 2 and 5 mg/kg/day of imidapril for 8 weeks revealed a dose-dependent antihypertrophic effect on the heart, while their hypotensive effects were moderate. In contrast, amlodipine showed only a very slight antihypertrophic effect on the heart, though it caused a much more evident reduction in BP compared to that in the imidapril-treated groups. These findings indirectly prove the recent hypothesis that the facilitated activity of renin-angiotensin system (RAS) in SHRs is presumably a more important factor for the regression of cardiac hypertrophy than the reduction of BP (afterload) itself (23, 24).

Molecular biological changes of the hypertrophied heart have been studied in rats in relation to RAS-dependent hypertensive models (25–27). In the hypertrophied left ventricle of 28-week-old SHR, mRNA levels for skeletal α-actin, atrial natriuretic peptide (ANP) and collagen (mainly type I and III) were increased. Moreover, the ratio of β- to α-myosin heavy chain (β- and α-MHC) mRNA was dramatically increased (25). Similar changes were observed in the heart of 24-week-old stroke-prone SHRs (SHRSP), a substrain of SHR (26). Additionally, in the study with SHRSP, the mRNA level for transforming growth factor-β_1 (TGF-β_1), an initial mediator that produces interstitial components such as collagen or fibronectin, was also increased. Skeletal α-actin and β-MHC are recognized as fetal phenotypes of contractile protein, and ANP is also known to be higher in the fetal heart. β-MHC has lower ATPase activity than α-MHC and has a slower shortening velocity. These results suggest a qualitative shift of myocardium to the fetal phenotypes in SHR and SHRSP. Furthermore, Kim et al. reported that continuous infusion of Ang II by the use of an osmotic minipump (200 ng/min) into Wistar rats caused similar changes in mRNA levels for TGF-β_1, collagen and skeletal α-actin, and increased the mRNA level for β-MHC and resulted in an increase of the ratio of β- to α-MHC (27). Additionally, TCV-116, a non-peptide selective angiotensin type 1 receptor antagonist completely abolished these changes in mRNA levels. These findings suggest that Ang II mediates molecular changes in hypertrophied myocardium. Effects of imidapril and amlodipine on the above mentioned mRNA levels have been also studied in SHR or SHRSP. Oral administration of imidapril (5 mg/kg/day) for 7 days decreased mRNA levels for ANP and collagen, and it increased the mRNA level for α-MHC in the left ventricle of 28-week-old SHRs to levels near those in WKY. In contrast, amlodipine

failed to suppress the mRNA levels for ANP and failed to increase the level for α-MHC, and suppression of the collagen gene expression was very weak. These reports may explain the different antihypertrophic activities of these two drugs in the present study.

Increased coronary flow and reduced coronary resistance in the imidapril-treated groups were observed. It has been reported that myocardial capillary growth in SHRs is reduced at the hypertensive stage (28, 29). The elevation of coronary vascular resistance (30) and reduction of coronary reserve (31) have also been reported. Improvement of coronary circulation by ACEIs, trandpril and cilazapril have been reported (30, 31). Clozel et al. demonstrated that chronic treatment with cilazapril prevented the reduction of coronary vascular reserve, increased capillary density and decreased the thickness of the wall of coronary arterioles in SHRs even when treatment was started after the establishment of hypertension (3-month-old) (31). In the present study, imidapril increased coronary flow, probably through a similar mechanism to that in other ACEIs. Moreover, the lower heart weights in imidapril-treated groups than those in other groups led to the elevated CoF/VW in SHR. The elevated CoF/VW may result in the enhancement of the oxygen supply in the heart accompanied by the improvement of cardiac function.

It is still controversial whether or not amlodipine has an antihypertrophic effect. It has been reported that the treatment of 8-week-old SHRs with amlodipine for 30 weeks reduced the heart weight (15). However, it has also been reported that an 8-week treatment with amlodipine in SHRs of the same age did not reduce ventricular weight (18). In the present study, total ventricular weight in the amlodipine-treated group was slightly but significantly lower than that in the vehicle-treated group. However, the LV weight including the septum was not significantly reduced. Thus, the antihypertrophic activity of amlodipine in the heart seems to be weak, and chronic treatment is probably necessary to expect the improvement or prevention of hypertension-associated cardiac hypertrophy. Additionally, it has been reported recently that the treatment with a high dose of amlodipine (above 15 mg/kg) markedly increased plasma renin activity in normotensive rats (32). Therefore, the antihypertrophic activity of amlodipine might be masked by the activated RAS in the present study.

Ca^{2+} channel antagonists, including amlodipine, have been known as strong vasodilators and widely used for the therapy of angina in the clinical stage. In the present study, however, amlodipine failed to increase coronary flow, and the flow at each preload level was similar to that in the vehicle-treated group. We examined the function of the isolated heart approximately 24 hr after the withdrawal of the dosing. The effects of amlodipine may have weakened prior to the examination. The present results therefore reflect not the acute but the chronic effects of amlodipine.

The effects of amlodipine on the coronary capillary growth in SHRs have not been reported. On the other hand, the improvement of coronary circulation by other DHP Ca^{2+} channel antagonists has been observed in parallel with the regression of the cardiac hypertrophy. Amann et al. reported that the treatment of SHRSPs with nifedipine (27 mg/kg/day) for 3 months, when the treatment was started from 6 months of age, significantly reduced blood pressure and heart weight and normalized capillarization to the level observed in age-matched WKY (28). On the other hand, Rakusan et al. reported that a 27-week treatment with nifedipine which was started at the 24 weeks of age in SHRs slightly reduced BP and HR, and inhibition of the capillary reduction was also very slight (29). Moreover, in 26-week-old female SHRs, treatment with felodipine for 13 weeks normalized BP, while the drug did not reduce the relative weight of left ventricular to body weight nor improved the capillary density in the LV (33). In the present study, coronary flow in the amlodipine-treated group at 5 and 10 cmH_2O of preload was similar to that in the group treated with imidapril at 2 mg/kg. However, CoF/VW in the amlodipine-treated group was less than that in the imidapril-group, because the antihypertrophic effect of amlodipine was very weak. Further continued and longer-lasting treatment may be necessary to attain an improvement of coronary circulation with amlodipine alone.

Imidapril dose-dependently reduced ventricular weight, whereas the reduction in the collagen content was not dose-dependent. Imidapril did not alter myocardial collagen concentration as compared with that in the vehicle- and the amlodipine-treated groups. Similar changes of the myocardial collagen concentration have also been previously reported in other ACEIs (21, 30, 34). In general, the half-life of collagen has been considered to be very long, around one year, because collagenase activity in tissues is nil or very low. Thus, longer treatment may be necessary to expect clear effects of ACEIs on the collagen metabolism. It is also generally accepted that the progress of cardiac hypertrophy is accompanied by the synthesis not only of interstitial collagen but also of the contractile proteins (7–9). Imidapril seems to inhibit collagen synthesis similarly at both doses and to inhibit myocyte hypertrophy at the higher dose.

The isolated hearts of the imidapril-treated animals exhibited better cardiac compliance than those of the vehicle-treated group. In contrast, amlodipine did not show any improvement in cardiac function, although the drug slightly lowered heart weight. Suppressed LV function

has been reported in 15-week-old SHRs. Rodrigues and McNeel studied the influences of streptozocin-induced diabetes on the LV function in a working heart (35). In this report, the increase of the LV pressure by the elevation of left atrial filling pressure were significantly reduced in control (non-diabetes) SHR compared to that in control WKYs, suggesting deterioration of contractile function of the heart.

In the hypertrophied heart, the increased amount of interstitial fibroblasts makes myocardium stiffer. This change has been supposed to reduce diastolic volume and thereby ventricular diastolic pressure might be elevated (36). In fact, at each preload level, LVEDP in the groups treated with imidapril was lower than that in the vehicle- and the amlodipine-treated group. From these findings and the above-mentioned previous reports (25–27), it is possible that imidapril increases cardiac output by improving the diastolic compliance of the ventricle through the reduction of the myocardial collagen content and by inhibiting the changes in contractile molecules. Moreover, it is possible that amlodipine failed to improve cardiac function because the drug neither inhibited collagen synthesis nor prevented molecular changes in contractile protein in SHR.

In conclusion, the 8-week treatment of imidapril demonstrated an evident antihypertrophic activity on the hearts of SHRs at the hypertensive stage. In these animals, improved function of the heart was confirmed, and coronary flow in the isolated heart is increased. In contrast, the 8-week treatment of amlodipine did not show any favorable activity on the cardiac function, although its hypotensive activity was stronger than that of imidapril. From these results in SHRs, it is indicated that ACEIs may be more favorable in the treatment of cardiac hypertrophy and deteriorated function than Ca^{2+} antagonists.

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REFERENCES

1 Tomanek RJ and Whitaker MT: Compensated function in hypertrophied ventricles of Wistar Kyoto and spontaneously hypertensive rats. Cardiovasc Res **24**, 204–209 (1990)
2 Gaasch WH: Diastolic dysfunction of the left ventricle: importance to the clinician. Adv Intern Med **35**, 311–340 (1990)
3 Hallbäck M, Isaksson O and Noresson E: Consequences of myocardial structural adaptation on left ventricular compliance and the Frank-Starling relationship in spontaneously hypertensive rats. Acta Physiol Scand **94**, 259–270 (1975)
4 Ketelhut R and Messerli FH: Hypertension: left ventricular hypertrophy, ventricular ectopy, and sudden death. Prim Care **18**, 577–592 (1991)
5 Strauer BE: The significance of coronary reserve in clinical heart disease. J Am Coll Cardiol **15**, 775–783 (1990)
6 Dahlöf B, Pennert K and Hansson L: Reversal of left ventricular hypertrophy in hypertensive patients. A metaanalysis of 109 treatment studies. Am J Hypertens **5**, 95–110 (1992)
7 Sadoshima J and Izumo S: Molecular characterization of angiotensin II-induced hypertrophy of cardiac myocytes and hyperplasia of cardiac fibroblasts. Critical role of the AT_1 receptor subtype. Circ Res **73**, 413–423 (1993)
8 Weber KT, Sun Y and Guarda E: Structural remodeling in hypertensive heart disease and the role of hormones. Hypertension **23** (Part 2), 869–877 (1994)
9 Schorb W, Booz GW, Dostal DE, Conrad KM, Chang KC and Baker KM: Angiotensin II is mitogenic in neonatal rat cardiac fibroblasts. Circ Res **72**, 1245–1254 (1993)
10 Kubo M, Kato J, Ochiai T and Ishida R: Pharmacological studies on (4*S*)-1-methyl-3-{(2*S*)-2-[*N*-((1*S*)-1-ethoxycarbonyl-3-phenylpropyl)amino]propionyl}-2-oxo-imidazolidine-4-carboxylic acid hydrochloride (TA-6366), a new ACE inhibitor: I. ACE inhibitory and anti-hypertensive activities. Jpn J Pharmacol **53**, 201–210 (1990)
11 Kubo M, Ochiai T, Kato J and Ishida R: Pharmacological studies on TA-6366, a new ACE inhibitor: II. Effect of long-term administration from the pre-hypertensive stage on blood pressure, relative heart weight and ACE activity of various tissues in spontaneously hypertensive rats (SHRs). Jpn J Pharmacol **57**, 517–526 (1991)
12 Nishiyama S, Kanno K, Yoneda H, Yano K and Yamaguchi I: Effects of the new angiotensin-converting enzyme inhibitor imidapril on renal hemodynamics and function in anesthetized dogs. Arzneimittelforschung **42**, 451–456 (1992)
13 Sugaya T, Minobe S, Taniguchi T, Hashimoto Y, Kubo M and Watanabe T: Studies on angiotensin I converting enzyme (ACE) inhibitory effect of imidapril. (I). Inhibition of various tissue ACEs in vitro. Folia Pharmacol Jpn (Nippon Yakurigaku Zasshi) **100**, 39–45 (1992) (Abstr in English)
14 Narita H, Kaburaki M, Doi H, Ogiku N, Yabana H, Kurosawa H and Ohmachi Y: Prolonging action of imidapril on the lifespan expectancy of cardiomyopathic hamsters. J Cardiovasc Pharmacol **27**, 861–871 (1996)
15 Ohtsuka M, Sakai S, Miura S, Kurosaki M, Koibuchi Y, Ono T and Shibayama F: Effects of nilvadipine, a new calcium entry blocker, on systemic blood pressure, cardiac hypertrophy and venous distensibility in spontaneously hypertensive rats. Arch Int Pharmacodyn Ther **301**, 228–245 (1989)
16 Saragoca MA, Portela JE, Abreu P, Plavnik F, Ventura R, Vanneta A, Ajzen H and Ramos OL: Reversal of left ventricular hypertrophy following treatment of hypertension with isradipine. J Cardiovasc Pharmacol **23**, Suppl 3, S28–S30 (1991)
17 Abernethy DR: Pharmacokinetics and pharmacodynamics of amlodipine. Cardiology **1**, 31–36 (1992)
18 Nayler WG: The effect of amlodipine on hypertension-induced cardiac hypertrophy and reperfusion-induced calcium overload. J Cardiovasc Pharmacol **12**, Suppl 7, S41–S44 (1988)
19 Patel VB, Siddiq T, Richardson PJ and Preedy VR: Protein synthesis in the hypertrophied heart of spontaneously hypertensive rats and comparison of the effects of an ACE-inhibitor and a calcium antagonist. Cell Biochem Funct **13**, 111–124 (1995)
20 Kojima M, Shiojima I, Yamazaki T, Komuro I, Zou Z, Wang

Y, Mizuno T, Ueki K and Tobe K: Angiotensin II receptor antagonist TCV-116 induces regression of hypertensive left ventricular hypertrophy in vivo and inhibits the intracellular signaling pathway of stretch-mediated cardiomyocyte hypertrophy in vitro. Circulation **89**, 2204–2211 (1994)

21 Mukherjee D and Sen S: Collagen phenotypes during development and regression of myocardial hypertrophy in spontaneously hypertensive rats. Circ Res **67**, 1474–1480 (1990)

22 Neely J, Lieber M, Battersby E and Morgan H: Effect of pressure development on oxygen consumption by isolated rat heart. Am J Physiol **212**, 804–814 (1967)

23 Black MJ, Bertram JF and Johnston CI: Cardiac growth during high and low dose perindopril treatment in spontaneously hypertensive rats. Clin Exp Pharmacol Physiol **23**, 605–607 (1996)

24 Reddy DS, Singh M, Ghosh S and Ganguly NK: Role of cardiac renin-angiotensin system in the development of pressure-overload left ventricular hypertrophy in rats with abdominal aortic constriction. Mol Cell Biochem **155**, 1–11 (1996)

25 Ohta K, Kim S and Iwao, H: Role of angiotensin-converting enzyme, adrenergic receptors, and blood pressure in cardiac gene expression of spontaneously hypertensive rats during development. Hypertension **28**, 627–634 (1996)

26 Kim S, Ohta K, Hamaguchi A, Yukimura T, Miura K and Iwao H: Effects of an AT_1 receptor antagonist, an ACE inhibitor and a calcium channel antagonist on cardiac gene expressions in hypertensive rats. Br J Pharmacol **118**, 549–556 (1996)

27 Kim S, Ohta K, Hamaguchi A, Yukimura T, Miura K and Iwao H: Angiotensin II induces cardiac phenotypic modulation and remodeling in vivo in rats. Hypertension **6**, 1252–1259 (1995)

28 Amann K, Greber D, Gharehbaghi H, Wiest G, Lange B, Ganten U, Mattfeldt T and Mall G: Effects of nifedipine and moxonidine on cardiac structure in spontaneously hypertensive rats. Stereological studies on myocytes, capillaries, arteries, and cardiac interstitium. Am J Hypertension **5**, 76–83 (1992)

29 Rakusan K, Cicutti N, Kazda S and Turek Z: Effect of nifedipine on coronary capillary geometry in normotensive and hypertensive rats. Hypertension **24**, 205–211 (1994)

30 Amrani FC, Cheaw SL, Chevalier B, Paolaggi F, Jouquey S, Hamon G and Swynghedauw B: Regression of left ventricular hypertrophy by converting enzyme inhibition in 12–15-month-old spontaneously hypertensive rats: effects on coronary resistance and ventricular compliance in normoxia and anoxia. J Cardiovasc Pharmacol **23**, 155–165 (1994)

31 Clozel JP, Kuhn H and Hefti F: Effects of chronic ACE inhibition on cardiac hypertrophy and coronary vascular reserve in spontaneously hypertensive rats with developed hypertension. J Hypertens **7**, 267–275 (1989)

32 Schricker K, Hamann M, Macher A, Kramer BK, Kaissling B and Kurtz A: Effect of amlodipine on renin secretion and renin gene expression in rats. Br J Pharmacol **119**, 744–750 (1996)

33 Wåhlander H, Nordborg C, Nordlander M and Friberg P: Functional and stereologic estimations of myocardial capillary exchange capacity in treated and untreated spontaneously hypertensive rats. Acta Physiol Scand **146**, 165–175 (1992)

34 Yonezawa T, Umemoto S, Fujii A, Katayama K and Matsuzaki M: Comparative effects of type 1 angiotensin ii receptor blockade with angiotensin-converting-enzyme inhibitor on left ventricular distensibility and collagen metabolism in spontaneously hypertensive rats. J Cardiovasc Pharmacol **27**, 119–124 (1996)

35 Rodrigues B and McNeel JH: Cardiac function in spontaneously hypertensive diabetic rats. Am J Physiol **251**, H571–H580 (1986)

36 Schraeger JA, Canby CA, Rongish BJ, Kawai M and Tomanek RJ: Normal left ventricular diastolic compliance after regression of hypertrophy. J Cardiovasc Pharmacol **23**, 349–357 (1994)