Cardioprotective Effects of Vasopeptidase Inhibition vs. Angiotensin Type 1-Receptor Blockade in Spontaneously Hypertensive Rats on a High Salt Diet

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The aim of our study was to compare the cardioprotective effects of vasopeptidase inhibition with those of angiotensin type 1 (AT₁)-receptor blockade, a diuretic and the combination of AT₁-receptor blockade and a diuretic in an experimental rat model of essential hypertension on a high salt diet. Spontaneously hypertensive rats (SHR) (n=73) were divided into 6 groups to receive the following diet and drug regimens for 8 weeks: 1) low salt controls (NaCl 0.5%); 2) high salt controls (NaCl 6%); 3) omapatrilat (40 mg/kg/d) on a high salt diet; 4) losartan (30 mg/kg/d) on a high salt diet; 5) hydrochlorothiazide (HCTZ; 10 mg/kg/d) on a high salt diet; and 6) losartan + HCTZ (30 + 10 mg/kg/d) on a high salt diet. Blood pressure was measured by tail-cuff plethysmography. The histological score of myocardial damage, myocardial collagen volume fraction (CVF), connective tissue growth factor (CTGF) expression and cardiomyocyte apoptosis were determined. As an antihypertensive, omapatrilat showed greater efficacy than monotherapy with losartan or HCTZ, and was equally effective as the combination of losartan + HCTZ. Assessed by myocardial damage score, omapatrilat and losartan protected cardiac morphology better than HCTZ or the drug combination. Omapatrilat decreased CVF to a greater extent than the other therapies, whereas losartan was most effective in decreasing CTGF expression. All drug treatments, except HCTZ, decreased cardiomyocyte apoptosis. Our findings provide evidence that both vasopeptidase inhibition and AT₁-receptor blockade exert cardioprotective properties beyond their blood pressure-lowering effects. Cardioprotection was associated with prevention of cardiomyocyte apoptosis and inhibition of extracellular matrix formation. (Hypertens Res 2004; 27: 609–618)

Key Words: hypertension, cardiac collagen, apoptosis, vasopeptidase inhibition, angiotensin type 1-receptor blockade

Introduction

In addition to hypertension-induced pressure overload imposed on the myocardium, endocrine, paracrine and autocrine factors such as cytokines, growth factors and vasoactive hormones are involved in the pathogenesis of cardiac hypertrophy and fibrosis associated with hypertensive heart disease.
disease (1–5). The main effector of the renin-angiotensin system (RAS), angiotensin II (Ang II), has been shown to act as a growth factor that regulates cell growth/apoptosis and collagen turnover (5). Locally synthesized Ang II may induce myocyte growth and fibrosis in the heart even without the influence of hemodynamic changes (3, 6).

The myocardial collagen synthesis is increased in adult spontaneously hypertensive rats (SHR) (7). Previous studies in SHR have revealed that both angiotensin converting enzyme (ACE) inhibitors and angiotensin type 1 (AT1)-receptor blockers (ARBs) prevent the development of left ventricular hypertrophy (LVH) and cardiac fibrosis (8) and also induce regression of myocardial damage despite an incomplete control of blood pressure (7, 9, 10). These findings suggest an important role for locally synthesized Ang II in the regulation of cardiac cell growth and collagen synthesis in this genetic model of hypertension (11, 12).

Previous studies indicate that Ang II indirectly regulates cardiac fibroblast function via specific growth factors (13). The cardiovascular and renal effects of Ang II are mediated primarily by AT1-receptors (14), and the Ang II-induced extracellular matrix production seems to be mediated mainly by transforming growth factor β (TGF-β) (5, 15, 16). We have recently shown in transgenic rats harboring human renin and angiotensinogen genes (17) that Ang II induces the myocardial expression of connective tissue growth factor (CTGF), a downstream effector of TGF-β profibrotic activities (18–21). Expression of CTGF has been reported in a variety of fibrotic disorders, such as cardiac and renal fibrosis (17, 18, 21–23); moreover, the CTGF expression appears to correlate closely with the degree of fibrosis (22).

Continuous loss of cardiomyocytes by apoptosis has been implicated in cardiac remodeling and development of heart failure. Increased cardiomyocyte apoptosis has been demonstrated in the hypertrophied left ventricle of SHR (24, 25) and in patients with essential hypertension (26, 27). Diez et al. (28) have shown that increased cardiomyocyte apoptosis in SHR is related in a temporal manner to local ACE activity independently of blood pressure level. Furthermore, treatment with an ACE-inhibitor or an ARB has been shown to prevent cardiomyocyte apoptosis (28, 29), indicating that Ang II is involved in the pathogenesis of cardiomyocyte apoptosis (30).

Vasopeptidase inhibitors (VPIs), which simultaneously inhibit ACE and neutral endopeptidase (NEP), represent a novel class of antihypertensive drugs. The antihypertensive effect of VPI has been demonstrated in several models of hypertension independently of the renin- and salt-status (31–34). The endogenous natriuretic peptides counteract the detrimental effects of Ang II in the heart. The VPIs increase the local effects of natriuretic peptides by blocking the NEP-mediated peptide metabolism and may therefore exert increased antiproliferative and antihypertrophic actions, and thus improve end organ protection beyond their blood pressure-lowering effect.

The aim of our study was to investigate whether we can achieve a better tissue protection with the VPI omapatrilat than with the clinically established antihypertensive treatments with the diuretic hydrochlorothiazide (HCTZ), the ARB losartan, or their combination. Furthermore, because high salt intake is a common problem in clinical practice, the cardiovascular effects of these drugs were investigated in SHR on a high salt diet. We hypothesized that omapatrilat is effective as an antihypertensive and has superior cardioprotective effects during conditions of sodium loading, when drugs inhibiting the RAS are usually not effective.

Methods

Experimental Animals, Drug Treatments and Sample Preparation

Male SHR were obtained from Harlan Sprague Dawley (Indianapolis, USA). The rats were housed at 23–25°C under a 12-h light/dark cycle with free access to rat chow and normal tap water. All the experimental procedures were approved by the Animal Ethics Committee of the University of Helsinki. At the beginning of the experiment the rats were 8–9 weeks old and weighed 190–260 g.

The rats received powdered rat chow containing 6% sodium chloride (Altromin Gesellschaft fur Tierernahrung mbH, Lage, Germany) for an acclimation period of 10 days. A control group received powdered rat chow with low salt concentration (0.5% NaCl).

After the acclimation period the SHR were divided into 6 groups (n = 11–13 animals in each group) to receive the following diet and drug regimens for 8 weeks: 1) low salt diet controls (NaCl 0.5%); 2) high salt diet controls (NaCl 6%); 3) omapatrilat (40 mg/kg/d) on a high salt diet; 4) losartan (30 mg/kg/d) on a high salt diet; 5) HCTZ (10 mg/kg/d) on a high salt diet; and 6) losartan + HCTZ (30 + 10 mg/kg/d) on a high salt diet.

Omapatrilat was provided by Bristol-Myers-Squibb Pharmaceuticals (Princeton, USA) and losartan by Merck-Sharp-Dohme (Rahway, USA), and HCTZ was obtained from Sigma Chemical Co. (St. Louis, USA). The doses of the drugs were chosen on the basis of our earlier study of omapatrilat (35) and other previously published experimental works (32, 34, 36–38). The actual doses were calculated by measuring the daily food consumption over the 5 days before the medications started, and then adjusted during the treatment period by remeasuring the food consumption.

Systolic blood pressure (SBP) was measured biweekly in conscious lightly restrained rats by tail-cuff plethysmography using a Doppler ultrasonic flowmeter to detect the pulse. SBP was measured an average of 3–5 times for each rat at each measurement. The body weights were recorded weekly throughout the study period.

After the 8-week treatment period the rats were sacrificed by intraperitoneal administration of phenobarbital.
Cardiomyocyte apoptosis was assessed by the terminal deoxynucleotide transferase-mediated ddUTP nick end labeling (TUNEL) assay as described previously (40, 41). In brief, nuclear DNA strand breaks were end-labeled with digoxigenin-conjugated deoxy-UTP by terminal transferase and visualized immunohistochemically with digoxigenin antibody conjugated to alkaline phosphatase. The assay was standardized with the use of adjacent tissue sections treated with DNase I to induce DNA fragmentation as a positive control of apoptosis. The percentage of TUNEL-positive cardiomyocytes was calculated in each animal (n = 9–12/group) in a transverse left ventricle tissue section under a microscope (×200 magnification) with an ocular grid. An average of 140 fields each containing an average of 125 myocyte nuclei were studied. Cardiomyocytes were identified by the presence of myofilaments surrounding the nucleus.

**Collagen Volume Fraction (CVF)**

CVF was determined by the picrosirius red method from three different slices of Direct Red 80 (Fluka Chemie, Buchs, Switzerland)-stained left ventricular sections under polarized light. Areas of connective tissue network and myocytes were quantified by a semi-automated computer-based analysis system as described by Brooks et al. (8). The results are presented as a percentage of collagen positive area per total myocardial area.

**Immunohistochemistry**

The expression of CTGF in the heart was determined by methods described elsewhere (12). Primary antibodies against rat CTGF (anti-rat CTGF; Abcam, Cambridge, UK) were used. The intensity of CTGF expression was evaluated by scoring the samples from 0 to 4 according to the amount of antibody positive labeling (n = 6–8).

**Apoptosis**

Cardiomyocyte apoptosis was assessed by the terminal deoxynucleotide transferase-mediated ddUTP nick end labeling (TUNEL) assay as described previously (40, 41). In brief, nuclear DNA strand breaks were end-labeled with digoxigenin-conjugated deoxy-UTP by terminal transferase and visualized immunohistochemically with digoxigenin antibody conjugated to alkaline phosphatase. The assay was standardized with the use of adjacent tissue sections treated with DNase I to induce DNA fragmentation as a positive control of apoptosis. The percentage of TUNEL-positive cardiomyocytes was calculated in each animal (n = 9–12/group) in a transverse left ventricle tissue section under a microscope (×200 magnification) with an ocular grid. An average of 140 fields each containing an average of 125 myocyte nuclei were studied. Cardiomyocytes were identified by the presence of myofilaments surrounding the nucleus.

**Plasma Renin Activity (PRA) and Plasma Aldosterone**

PRA (42) and plasma aldosterone concentration (43) were measured by radioimmunoassay as described previously.

**Quantitative in Vitro Autoradiography of ACE**

Quantitative in vitro autoradiography of cardiac ACE was performed using the radioligand 125I-MK351A as described previously (35, 44, 45). The optical densities were quantified by an AIDA computer image analyzing system (AIDA 2D densitometry) coupled to the FUJIFILM BAS-5000 phosphoimager (Tamro, Finland) (35). Specific binding was calculated as total binding minus nonspecific binding.

**Statistical Analyses**

Data were analyzed by ANOVA with the Statview 5 program. Comparisons of group means were performed by Fisher’s post-hoc-analyses. When the data consisted of repeated measures at successive time points, ANOVA for repeated measures was used. Values of p<0.05 were considered to indicate statistical significance. Data are shown as the mean ± SEM unless otherwise indicated.

**Results**

**SBP**

In the beginning of the experiment, SBP in the SHR averaged 154 ± 3.1 mmHg. There were no statistically significant differences in SBP among the groups. After 10 days with different salt diets, the blood pressure-levels in the high salt groups were significantly elevated compared to those of the low salt control group. A high salt diet for 10 weeks significantly increased the blood pressure (Fig. 1A). Before the medication started there still were no significant differences among the groups that included a high salt diet. All drug treatments decreased the blood pressure significantly com-
pared to the controls during the high salt diet (Fig. 1B). At the end of the experiment, the antihypertensive effect of omapatrilat was equal to that of the combination of losartan + HCTZ (\( p < 0.001 \)) and more pronounced compared to monotherapy with losartan or HCTZ (\( p < 0.001 \), respectively).

Table 1.  Body Weight and Relative Cardiac Weight (Heart Weight to Body Weight) Were Measured at the End of the Experiment

<table>
<thead>
<tr>
<th>Group</th>
<th>Body weight (g)</th>
<th>Relative cardiac weight (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5% NaCl control</td>
<td>330 ± 7</td>
<td>3.8 ± 0.07</td>
</tr>
<tr>
<td>6% NaCl control</td>
<td>328 ± 4</td>
<td>4.3 ± 0.07 **</td>
</tr>
<tr>
<td>6% NaCl + oma</td>
<td>315 ± 4</td>
<td>3.9 ± 0.08 **</td>
</tr>
<tr>
<td>6% NaCl + los</td>
<td>331 ± 6</td>
<td>4.0 ± 0.06 ▶</td>
</tr>
<tr>
<td>6% NaCl + HCTZ</td>
<td>319 ± 5</td>
<td>4.0 ± 0.07 **</td>
</tr>
<tr>
<td>6% NaCl + (los + HCTZ)</td>
<td>321 ± 5</td>
<td>3.5 ± 0.07 **</td>
</tr>
</tbody>
</table>

Values are given as mean ± SEM. oma, omapatrilat; los, losartan; HCTZ, hydrochlorothiazide. * \( p < 0.05 \) and *** \( p < 0.001 \) as compared to the 0.5% NaCl controls. ▶ \( p < 0.05 \), ▶▶ \( p < 0.01 \) and ▶▶▶ \( p < 0.001 \) compared to the 6% NaCl controls.

**Heart Weight and Body Weight**

The high sodium diet induced cardiac hypertrophy as assessed by the heart weight to body weight ratio (Table 1). The relative cardiac weight was 4.3 mg/g in the controls on a high salt diet compared to 3.8 mg/g in the low salt diet controls (\( p < 0.001 \)). All drug treatments significantly lowered the relative cardiac weight in comparison to that of the controls on a high salt diet, but combination treatment was the most efficient of the drugs studied. There was no difference among the groups in the body weight gain.

**Cardiac Morphology**

Cardiac samples from the high salt control group had significantly more pathological changes in comparison with all the other groups (Fig. 2). The large epicardial arteries showed marked adventitial fibrosis and thickening of the medial layer. There were also some inflammatory cells in the perivascular areas. The small intramuscular arteries showed variable amounts of concentric hypertrophy narrowing the luminal space. In the cardiac muscle there were numerous scars indicating previous infarcts. Omapatrilat and losartan decreased the myocardial damage score to the same level found in the low salt SHR controls (Fig. 3). In contrast, HCTZ alone or in combination with losartan conferred only partial protection against salt-induced myocardial damage.

**CVF**

The high salt diet increased the amount of interstitial collagen deposition and fibrosis in SHR (\( p < 0.01 \)) compared to the low salt controls (Fig. 4). All drug treatments prevented the development of cardiac fibrosis. However, treatment with omapatrilat was most effective in decreasing the CVF.
Fig. 2. Sections of ventricular tissue were stained with the Masson’s trichrome technique. Each sample was scored from 0 to 3 according to morphological changes (as described in the text) to determine the cardiac morphological score. The low salt control group and the losartan- and omapatrilat-treated groups (A, C, D, respectively) showed normal cardiac histology, whereas the high salt controls had marked perivascular fibrosis extending also into the interstitial areas (B). Magnification: ×200.

Fig. 3. Cardiac morphology score after 8 weeks of medication compared to that in the non-treated control rats. The high dietary salt was associated with significantly more pathological changes in cardiac structure compared to the low salt diet controls. Omapatrilat and losartan almost completely prevented the development of pathological lesions, while the effect of HCTZ or the combination of losartan and HCTZ was less significant. * p<0.05, ** p<0.01, *** p<0.001 vs. the 0.5% NaCl controls. † p<0.05, †† p<0.01 vs. the 6% NaCl controls.

Fig. 4. The amount of cardiac collagen after the 8-week treatment period as shown by collagen volume fraction (CVF) measurement. Treatment with omapatrilat significantly decreased the CVF in comparison to the other drugs studied. ** p<0.01 vs. the 0.5% NaCl controls. ‡ p<0.05, ‡‡‡ p<0.001 vs. the 6% NaCl controls.
CTGF Expression

The high salt diet increased the myocardial CTGF expression 2.25-fold (Fig. 5). The salt-induced increase in CTGF expression was prevented by all drug treatments. However, losartan decreased CTGF more effectively than omapatrilat or HCTZ (p<0.05 for both groups).

Apoptosis

The number of apoptotic cardiomyocytes increased by 125% in the high salt diet control group compared to the low salt diet controls (p<0.01) (Fig. 6). Omapatrilat, losartan, and the drug combination prevented the salt-induced increase in cardiomyocyte apoptosis, whereas HCTZ did not significantly influence the number of apoptotic cells.

PRA and Plasma Aldosterone

The high salt diet tended to decrease PRA, but this difference did not reach statistical significance (Table 2). Omapatrilat, losartan, and the drug combination increased PRA. The combination of losartan and HCTZ increased PRA more than omapatrilat. The high salt diet decreased the plasma aldosterone concentration by 50% in comparison to the low salt diet controls (p<0.05) (Table 2). Omapatrilat and losartan tended to decrease the plasma aldosterone level compared to the high salt control group, although this difference was not statistically significant. In contrast, HCTZ increased plasma aldosterone.

Fig. 5. Connective tissue growth factor (CTGF) score after the 8-week treatment period. The high dietary salt upregulated the expression of cardiac CTGF in comparison to that of the control group on a low salt diet. All drug treatments prevented CTGF upregulation compared to the 6% NaCl control group. The CTGF expression was significantly suppressed in the losartan-treated group compared to the omapatrilat- or the HCTZ-treated group. ** p<0.01 vs. the 0.5% NaCl controls. † p<0.05, ††† p<0.001 vs. the 6% NaCl controls.

Fig. 6. The effect of different medications on cardiomyocyte apoptosis in comparison to controls on a low or high salt diet. The amount of apoptosis in the control group on a high salt diet was increased compared with that in the low salt diet controls. All the drugs except for HCTZ decreased the cardiomyocyte apoptosis compared to the 6% NaCl control group. Omapatrilat and losartan reduced the amount of apoptosis most effectively, while HCTZ did not affect the number of apoptotic cells. There was no significant difference between omapatrilat and losartan treatment. ** p<0.01 vs. the 0.5% NaCl controls. † p<0.05, ††† p<0.001 vs. the 6% NaCl controls.

Table 2. Plasma Renin Activity (PRA), Cardiac Angiotensin Converting Enzyme (ACE) Inhibition and Plasma (P-) Aldosterone at the End of the Study

<table>
<thead>
<tr>
<th>Group</th>
<th>PRA (ng/ml)</th>
<th>Autoradiography of cardiac ACE (%)</th>
<th>P-aldosterone (µg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5% NaCl control</td>
<td>3.0 ± 0.4</td>
<td>100.0 ± 8.5</td>
<td>160.0 ± 31.7</td>
</tr>
<tr>
<td>6% NaCl control</td>
<td>1.0 ± 0.2</td>
<td>108.2 ± 13.6</td>
<td>79.1 ± 19.9*</td>
</tr>
<tr>
<td>6% NaCl + oma</td>
<td>18.6 ± 2.0</td>
<td>12.1 ± 1.0**</td>
<td>68.1 ± 4.7*</td>
</tr>
<tr>
<td>6% NaCl + los</td>
<td>20.8 ± 1.5</td>
<td>109.4 ± 11.9</td>
<td>64.1 ± 6.7**</td>
</tr>
<tr>
<td>6% NaCl + HCTZ</td>
<td>5.4 ± 1.0</td>
<td>96.5 ± 14.7</td>
<td>146.5 ± 44.3</td>
</tr>
<tr>
<td>6% NaCl + (los + HCTZ)</td>
<td>24.5 ± 1.7</td>
<td>65.7 ± 6.7**</td>
<td>101.2 ± 16.3</td>
</tr>
</tbody>
</table>

Values are given as mean ± SEM. oma, omapatrilat; los, losartan; HCTZ, hydrochlorothiazide. * p<0.05, ** p<0.01 and *** p<0.001 as compared to the 0.5% NaCl controls. ††† p<0.001 compared to the 6% NaCl controls.
Autoradiography of ACE

Autoradiography was used to determine the expression and activity of ACE in the heart. The high sodium diet did not influence cardiac ACE expression (Table 2). Omapatrilat inhibited cardiac ACE by 88%. In contrast, neither losartan nor HCTZ influenced cardiac ACE expression.

Discussion

The main findings of this study are: 1) the antihypertensive effect of the VPI omapatrilat was superior to that of the ARB losartan in SHR on a high salt diet; 2) both omapatrilat and losartan were equally effective in preventing the detrimental effects of dietary salt on myocardial morphology as well as in preventing a salt-induced increase in cardiomyocyte apoptosis; 3) as compared to losartan, omapatrilat was more effective in preventing extracellular matrix accumulation when assessed by myocardial collagen volume fraction; and 4) the drug combination of losartan and HCTZ decreased the blood pressure effectively, but its cardioprotective effect was less pronounced.

Previous studies have shown that a high salt intake elevates blood pressure and promotes cardiac fibrosis in SHR and also induces LVH and cardiac fibrosis in normotensive Wistar-Kyoto rats (WKY) (46, 47). The proposed mechanisms of this pressure-independent LVH are the extracellular volume expansion and the increased activity of the sympathetic nervous system associated with high sodium chloride intake (46). In addition, the high salt diet has been shown to induce overexpression of cardiac TGF-β mRNA in both SHR and WKY (47). In the present study the high dietary salt concentration was clearly associated with local pathological changes in cardiac structure. As expected, the high salt intake increased the blood pressure level. The high content of salt in the diet also increased the cardiac interstitial collagen deposition and fibrosis, significantly induced the expression of the fibrogenic and pro-apoptotic CTGF, and accelerated the rate of myocardial apoptosis.

The blood pressure-lowering efficacy of the VPI omapatrilat was greater than that by monotherapy with losartan or HCTZ, and was as effective as combination treatment with losartan and HCTZ. This is in agreement with previous studies showing that the antihypertensive efficacy of VPIs was superior to that of conventional treatments (48, 49). The simultaneous inhibition of NEP and ACE results both in increased availability of natriuretic peptides and bradykinin, and in reduced formation of Ang II, thereby decreasing vascular tone and lowering blood pressure by dual mechanisms (31, 49, 50). Under conditions of salt loading, most antihypertensives, and particularly drugs inhibiting the RAS, lose some of their efficacy due to the suppressed renin activity. If a diuretic is then added to the medication, the antihypertensive effect is usually regained, which is why the combination treatment was used in this study.

The high salt intake markedly induced cardiac hypertrophy, as assessed by the heart weight to body weight ratio. The antihypertrophic effects of all drug treatments were significantly greater than those in the high salt controls. The combination treatment, which together with omapatrilat was the most efficient as a blood pressure-lowering agent, was also the most effective in decreasing the relative cardiac weight.

Although omapatrilat reduced the blood pressure level more effectively than losartan, the beneficial effects on cardiac morphology did not differ between these drugs. Both provided almost complete protection against the development of pathological lesions. Thus, the AT1 blockade by losartan appears to have additional, blood pressure-independent, cardioprotective effects. Furthermore, losartan was more effective than omapatrilat in decreasing cardiac CTGF expression. CTGF has been reported to mediate the stimulatory actions of TGF-β on extracellular matrix synthesis (22) and to enhance the proliferation of fibroblasts and endothelial cells, as well as to induce apoptosis in vascular smooth muscle cells (51). On the other hand, omapatrilat was superior to losartan in decreasing the cardiac interstitial collagen content. The high dietary salt intake weakens the efficacy of most antihypertensives, including ARBs, while the VPIs retain their antihypertensive efficacy independently of the renin- and salt-status. The higher SBP level in the losartan-treated group may have resulted in the higher CVF compared to the omapatrilat-treated group.

Experimental and clinical findings indicate that loss of cardiomyocytes by apoptosis contributes to the myocordial remodeling seen in hypertensive heart disease (30, 52, 53). Ang II promotes both cardiac hypertrophy and apoptosis via the AT1-receptors in cardiomyocytes (30, 54). Long-term pharmacological intervention with an ACE inhibitor or an ARB prevents cardiomyocyte apoptosis in SHR (28, 29). In this study the rats on a high salt concentration diet had significantly more apoptotic cardiomyocytes than rats on a low salt diet. Both omapatrilat and losartan effectively reduced the degree of cardiomyocyte apoptosis to the level seen in the control group on the low salt concentration diet. Combination treatment also decreased cardiomyocyte apoptosis compared to that of the high salt control group, but the effect was not as obvious as with losartan alone. Even though combination treatment was as effective as omapatrilat in reducing blood pressure, there was a significant difference in favor of omapatrilat in the ability to prevent apoptosis. Thus, the prevention of cardiomyocyte apoptosis by different types of antihypertensive regimen is not directly connected to the blood pressure-lowering efficacy of the drugs, but rather to their ability to regulate apoptosis at the tissue level.

Surprisingly, the significant cardioprotective effect of losartan, as assessed by the cardiac morphological score, was impaired when using losartan in combination with HCTZ in spite of the improvement in blood pressure control. More-
over, losartan used as a monotherapy was more effective than combination treatment in reducing the expression of CTGF. There was no significant difference between losartan and the combination treatment in decreasing the interstitial collagen content. Earlier studies have given conflicting results in regard to the effect of HCTZ on myocardial fibrosis in SHR; the results range from prevention of cardiac hypertrophy and fibrosis (55) to increasing myocardial collagen concentration in HCTZ-treated SHR (56). In general, the conventional antihypertensive drugs, such as diuretics, seem to have minor antihypertrophic and antifibrotic effects on the heart in comparison to ACE inhibitors and ARBs (57, 58).

The reason for the less beneficial cardiac structural effects of the combination treatment with losartan and HCTZ compared to losartan monotherapy is not clear. Plasma renin activity was highest in rats receiving combination therapy, suggesting that HCTZ activated the RAS, which could counteract the effects of AT1-receptor blockade. On the other hand, cardiac ACE activity was down-regulated in rats receiving the combination of losartan and HCTZ. The aldosterone level in the HCTZ- and the combination-treated groups tended to be higher than in the groups receiving omapatrilat or losartan. It has recently been demonstrated both in animal and human studies that aldosterone is locally produced in the heart (59–61) and acts via mineralocorticoid receptors in the myocardium to enhance extracellular matrix and collagen deposition (62, 63). The differences in renin activity and aldosterone levels may at least partly explain the impairment of cardiac structure in diuretic-treated rats.

In conclusion, both omapatrilat and losartan exerted cardioprotective properties beyond the blood pressure-lowering effect in SHR with high salt intake. During the medication period there were no statistically significant differences between the group treated with omapatrilat and the group receiving the combination of losartan + HCTZ, and these groups attained the same blood pressure level at the end of the experiment. Thus, the difference in cardiac structural changes between the omapatrilat- and combination-treatment groups was attributed to effects of omapatrilat other than the blood pressure-lowering effect. When compared to monotherapy with losartan, omapatrilat was more effective as an antihypertensive and in decreasing collagen deposition, whereas losartan was superior to omapatrilat in preventing CTGF upregulation. Both drugs, however, beneficially prevented the development of cardiac lesions and cardiomyocyte apoptosis.

The present study underscores the importance of blockade of the RAS in the treatment of hypertensive heart disease. Theoretically, the VPIs offer an interesting approach to the therapeutic strategy for cardiovascular diseases. Still, as a consequence of the complex pharmacology of these drugs, more studies are needed to clarify the exact mechanisms and interactions of the dual ACE/NEP inhibition at the tissue level.

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