Effects of imidapril on NOS expression and myocardial remodelling in failing heart of Dahl salt-sensitive hypertensive rats

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Abstract

Objectives: To elucidate the relationship between renin–angiotensin system and nitric oxide in hypertensive heart failure, we evaluated the effects of long-term treatment with imidapril, an angiotensin-converting enzyme inhibitor, on endothelial-cell nitric oxide synthase (eNOS) and inducible NOS (iNOS) expression in the left ventricle (LV) and its relation to myocardial remodelling in failing heart of Dahl salt-sensitive hypertensive rats (DS) fed a high-salt diet. Methods: In DS rats fed an 8\% NaCl diet after the age of 6 weeks, a stage of concentric left ventricular hypertrophy at 11 weeks (DSLVH) was followed by a distinct stage of fatal left ventricular failure with chamber dilatation at 18 weeks (DSCHF). Imidapril (DSCHF-I, $n=7$, 1 mg/kg/day, subdepressor dose) or vehicle (DSCHF-V, $n=7$) were given from DSLVH to DSCHF stage for 7 weeks, and age-matched (18 weeks) Dahl salt-resistant rats fed the same diet were served as control group (DR-C, $n=7$). Results: Markedly increased left ventricular end-diastolic diameter and reduced fractional shortening in DSCHF-V was significantly ameliorated in DSCHF-I using transthoracic echocardiography. The level of eNOS mRNA and protein in the LV was significantly suppressed in DSCHF-V compared with DR-C, and significantly increased in DSCHF-I compared with DR-C and DSCHF-V. The iNOS mRNA and protein and the fibrosis factor expression of type I collagen mRNA were significantly increased in DSCHF-V compared with DR-C, and significantly decreased in DSCHF-I compared with DSCHF-V. DSCHF-V demonstrated a significant increase in wall-to-lumen ratio, perivascular fibrosis, and myocardial fibrosis. These changes in the microvasculature were improved significantly by imidapril. Conclusions: Subdepressor dose of imidapril may ameliorate the endothelial damage not only by inhibiting production of angiotensin II but also by promoting eNOS and inhibiting iNOS mRNA and protein expression in the LV, and this increased eNOS mRNA and protein level may have a role in the improvement of congestive heart failure and myocardial remodelling. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: ACE inhibitors; Gene expression; Heart failure; Nitric oxide; Remodelling

1. Introduction

Since nitric oxide (NO), an endothelium-dependent vasodilator, was identified by Moncada et al. [1], many studies have reported the importance of NO in circulatory regulation. With respect to circulatory actions alone, it has also been found that NO not only dilates blood vessels but also influences myocardial contractility [2]. Furthermore, some studies have indicated that expression [3] and activation [4] of inducible nitric oxide synthase (iNOS) are observed in patients with chronic heart failure. However, other studies have reported that these parameters vary among underlying diseases that caused heart failure [5], and a consensus has not been obtained. The renin–angiotensin system is also an important factor involved in circulatory regulation. Angiotensin II is known to exhibit cardiotonic actions directly on the myocardium [6,7], but has been reported to exhibit negative inotropic actions indirectly via NO [8].

With regard to chronic heart failure, many disease models have been studied. A hamster cardiomyopathy model [9] and dog or pig rapid pacing models [10] are known as congestive heart failure models with symptoms
such as pleural fluid, ascites and congestion. Recently, salt-loaded Dahl salt-sensitive (DS) rats were reported as a model that can be used relatively easily [11–14]. It has been shown that DS rats develop cardiac hypertrophy at 11 weeks of age and fatal heart failure with cardiac enlargement at 18 weeks of age when food containing 8% NaCl is given from 6 weeks of age. This model can be studied with respect to individual stages, since the reproducibility of deterioration of the condition is good.

As described above, changes in nitric oxide synthase (NOS) are observed in patients with chronic heart failure [3–5]. Chronic angiotensin-converting enzyme (ACE) inhibitor has been demonstrated to have favorable effects on left ventricular function and survival in patients with congestive heart failure. The institution of ACE inhibitor has been shown to improve left ventricular geometry, loading conditions, and neurohormonal status in both humans and animals with developing congestive heart failure [15,16]. Furthermore, it is known that ACE inhibitors reduce the formation of angiotensin II and prevent the degradation of bradykinin [17], an endothelium-dependent relaxant that works by stimulating the release of NO [18]. However, very few studies have evaluated whether the beneficial effects of ACE inhibitors on myocardial remodelling is associated with direct local gene expression of endothelial-cell NOS (eNOS) and iNOS mRNA and protein in failing heart of DS rats. The purpose of the present study was to evaluate the effects of long-term treatment with a subdepressor dose of imidapril, an ACE inhibitor, on expression of eNOS and iNOS mRNA and protein in the LV, and its relation to myocardial remodelling including type I collagen mRNA and cardiac function in failing hearts of DS rats.

2. Methods

2.1. Animal models and experimental designs

All procedures were performed in accordance with international standards on animal welfare. Male inbred DS and Dahl salt-resistant (DR) rats, which were originally obtained from Brookhaven National Laboratories, Upton, New York, were bred and supplied by Eisai Co., Ltd. (Tokyo, Japan) [19,20]. After they were weaned, the rats were fed a diet containing 0.3% NaCl until the age of 6 weeks. Thereafter, they were fed a diet containing 8% NaCl. The rats were weaned, and their systolic blood pressure (SBP) was measured by the tail-cuff method (Muromachi Kikai, model MK-1100, Tokyo, Japan) before feeding with the 8% NaCl diet and at 1-week intervals thereafter. Transthoracic echocardiography (model SSA-260A, Toshiba, Tokyo, Japan, with a 7.0/5.0-MHz micro probes, PVF-738H, Tokyo, Japan) was performed as described [11–14,21] on the day before the rats were killed. We determined the left ventricular end-diastolic diameter (EDD) and fractional shortening (FS) [11–14]. In DS rats fed an 8% NaCl diet after the age of 6 weeks, a stage of concentric left ventricular hypertrophy at 11 weeks (DSLHV) is followed by a distinct stage of fatal left ventricular failure with chamber dilatation at 18 weeks (DSCHF). Imidapril (DSCHF-I, n = 7, 1 mg/kg/day, sub-depressor dose, Tanabe Seiyaku Co., Ltd) or vehicle (DSCHF-V, n = 7) were given from the DSLHV to DSCHF stage in drinking water for 7 weeks, and age-matched DR rats (DR-C, 18 weeks, n = 7) fed the same diet were served as a control group. Fourteen DS rats at the stage of DSLHV were divided into two groups (DSCHF-V and DSCHF-I) randomly (protocol 1). Experimental designs of protocol 2 and 3 were treated in the same manner as described above in protocol 1.

2.2. Protocol 1

2.2.1. Reverse transcription-polymerase chain reaction (RT-PCR)

After 7 weeks of treatment, the rats were anesthetized with sodium pentobarbital (50 mg/kg IP) and decapitated, and the heart was immediately excised. The left ventricle was carefully separated from the atria and right ventricle, weighed, immediately frozen in liquid nitrogen, and stored at −80°C until extraction of total RNA. Total RNA was prepared as previously described [22]. RT-PCR was performed by standard methods with 1 μg of total RNA [23]. First-strand cDNA was synthesized with random primers and Molony murine leukemia virus reverse transcriptase (Promega, Madison, WI, USA). PCR amplification was then performed with synthetic gene-specific primers for eNOS (upstream primer, 5'-TCCAGTAACACAGACAGTGCA-3'; downstream primer, 5'-CAGGAAGTAGTGAGGAGC-3'; product length, 693 bp) [24], iNOS (upstream primer, 5'-GAGATCAATGACGACCT-3'; downstream primer, 5'-AGAATGGAGATAGGACGT-3'; product length, 217 bp) [25], and type I collagen (upstream primer, 5'-TGTTGCTGGTCTCAGGGTAG-3'; downstream primer, 5'-TTGTCGTAGCAGGGTCTT TTC-3'; product length, 254 bp) [26], using a DNA PCR kit (Perkin-Elmer, Norwalk, CT, USA) for 30 cycles of denaturation at 95°C for 30 s, annealing at 55°C for 30 s, and elongation at 72°C for 1 min. Parallel amplification of rat glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was performed for reference with primers as described [27]. Reaction conditions were optimized to obtain reproducible and reliable amplification within the logarithmic phase of the reaction, as determined by preliminary experiments. The reaction was linear to 35 cycles with use of the ethidium bromide detection method. PCR products were separated by electrophoresis on a 2% agarose gel containing ethidium bromide and were visualized by ultraviolet-induced fluorescence. The intensity of each band was quantified using a densitometer. The resulting densities of the eNOS, iNOS,
type I collagen bands were expressed relative to the corresponding densities of the GAPDH bands from the same RNA sample [28–30].

2.3. Protocol 2

2.3.1. Western blot analysis

2.3.1.1. Tissue preparation. LV was homogenized (25% w/v) in 10 mmol/l HEPES buffer, pH 7.4, containing 320 mmol/l sucrose, 1 mmol/l EDTA, 1 mmol/l DTT, 10 μg/ml leupeptin, and 2 μg/ml aprotinin at 0 to 4°C with a polytron homogenizer. Homogenate was centrifuged at 1000×g for 5 min at 4°C and the resulting supernatant was used as a post-nuclear fraction. Protein concentrations were determined with bovine serum albumin as a standard protein [31].

2.3.1.2. Western blotting. The post-nuclear fraction (eNOS: 50 μg of protein, iNOS: 200 μg of protein) of each sample was subjected to SDS–PAGE using 10% gels [32]. The proteins in the gels were transferred electrophoretically to PVDF sheets for 1 h at 2 mA/cm² as described [33]. The sheets were immunoblotted with an anti-eNOS or anti-Mac NOS antibody (Transduction Laboratories, anti-eNOS: N30020, anti-Mac NOS: N39120) in a buffer containing 10 mmol/l Tris/HCl, pH 7.5, 100 mmol/l NaCl, 0.1% Tween 20, and 5% skim milk followed by peroxidase-conjugated goat anti-mouse IgG (Amersham Life Science Inc.) [33]. The eNOS and iNOS proteins transferred to the sheets were detected using the ECL immunoblotting detection system (Amersham Life Science Inc.). The amount of each protein was quantified using a densitometer in a linear range and expressed as percent relative to that in the non-treated rat.

2.4. Protocol 3

2.4.1. Histologic examination and evaluation of myocardial remodelling

Histological examination was studied as described in detail previously [29,30,34]. Briefly, excised hearts were perfused with physiological saline solution containing adenosine 10 μg/kg and nitroglycerin 10 μg/kg and then with 6% formaldehyde solution via retrograde infusion into the ascending aorta at a pressure of 90 mm Hg. The LV was separated from the right ventricle, the atria, and the great vessels, and cut into five pieces perpendicular to the long axis. For light microscopy, 1.5-μm-thick sections were cut (microtome, Tokyo, Japan). Paraffin slices from each heart were mounted on glass slides and stained with hematoxylin–eosin and Masson’s trichrome stains. All histopathological sections of each animal were examined using a 3CCD color video camera (Sony, model DXC-930, Tokyo, Japan) mounted on a standard microscope (Olympus, BHS-F, Tokyo, Japan). Drawings of the limits of the vessels were made on the screen of a multiscan color computer display (Sony, model CPD-175SF7, Tokyo, Japan) and then digitized with a two-dimensional analysis system (Mac SCOPE, Mitani Corporation, Japan) connected with a Macintosh computer system (Power Macintosh G3, Apple Computer Inc.). Histopathological findings of the myocardium and coronary arterioles were examined. We always measured the capillary density and cross-sectional area in the endocardium of the posterior portion of the left ventricular free wall. In this part of the heart, shrinkage was minimal and orientation of the myocardial fibres was similar from one heart to another. We analyzed five sites from each ventricle in all rats. To assess thickening of the coronary arterial wall and perivascular fibrosis, the transsectional images of the area of the total small arteriolar lumen ≤10⁴ μm² were studied. The inner border of the lumen and the outer border of the tunica media were traced in each arterial image with hematoxylin–eosin staining at ×100 to ×400 magnification, and the areas encircled by the tracings were calculated. In quantification, nonround vessels resulting from oblique transection or branching were excluded, and only round vessels were studied. The wall-to-lumen ratio (the area of the vessel wall divided by the area of the total blood vessel lumen) was determined. The area of fibrosis immediately surrounding blood vessels was calculated, and perivascular fibrosis was determined as the ratio of the area of fibrosis surrounding the vessel wall to the total area of the vessel. To assess the area of myocardial fibrosis, the area of pathological collagen deposition was measured in the microscopic field of each Masson’s trichrome-stained section. The ratio of the total area of fibrosis within the left ventricular myocardium to the total area of the left ventricular myocardium in each heart was calculated and was used for analysis. The histopathology on the sections from each rat was carried out by an operator who was blinded to the treatment groups.

2.5. Statistical analysis

All results are expressed as mean±SEM. The mean values were compared among the three groups using ANOVA followed by the Bonferroni test. Differences of P<0.05 were considered statistically significant. Calculations, including those of derived values, and statistical tests, were performed using the appropriate software (Stat View-J 4.5, Abacus Concepts Inc., CA, USA) and a Power Macintosh computer system (G3, Apple Computer Inc.).

3. Results

3.1. Systemic hemodynamics, body weight, and left ventricular weight

As shown in Table 1, SBP in DSCHF-V and DSCHF-I
Table 1

<table>
<thead>
<tr>
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<th>Dahl salt-resistant rats</th>
<th>Dahl salt-sensitive rats</th>
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<tr>
<td></td>
<td>DR-C (n=7)</td>
<td>DSCHF-V (n=7)</td>
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<tr>
<td></td>
<td></td>
<td>DSCHF-I (n=7)</td>
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<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>127±3</td>
<td>251±6*</td>
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<tr>
<td></td>
<td></td>
<td>247±7*</td>
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<tr>
<td>Heart rate, bpm</td>
<td>436±12</td>
<td>434±10</td>
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<td></td>
<td></td>
<td>430±11</td>
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<tr>
<td>Body weight, g</td>
<td>427±8</td>
<td>294±4*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>386±5†</td>
</tr>
<tr>
<td>LV weight, mg</td>
<td>874±21</td>
<td>1348±25*</td>
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<td></td>
<td></td>
<td>1078±20*†</td>
</tr>
<tr>
<td>LV weight/body weight, mg/g</td>
<td>2.05±0.05</td>
<td>4.58±0.06*</td>
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<td>2.79±0.08**</td>
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*Values are mean±SEM. DR-C, Dahl-salt resistant rats (control group); DSCHF-V, failing heart of Dahl salt-sensitive rats treated with vehicle; DSCHF-I, failing heart of Dahl salt-sensitive rats treated with imidapril; LV, left ventricular.

was similar and significantly higher than that in DR-C. Heart rate was similar in DR-C and DSCHF-V, and was not changed by the administration of imidapril. Body weight in DSCHF-V was significantly decreased compared with DR-C, and significantly increased in DSCHF-I compared with DSCHF-V. The left ventricular mass of the DSCHF-V was significantly increased compared to that of DR-C in body weight-corrected values, and significantly decreased in DSCHF-I compared with DSCHF-V after 7 weeks treatment with imidapril.

3.2. Left ventricular EDD and FS

Left ventricular EDD in DSCHF-V was significantly increased compared with DR-C (9.44±0.38 vs. 7.40±0.07 mm, P<0.01), and significantly decreased in DSCHF-I compared with DSCHF-V (7.59±0.26 mm, vs. DSCHF-V, P<0.01) (Fig. 1A). Left ventricular FS in DSCHF-V was significantly decreased compared with DR-C (25.20±0.73 vs. 42.26±0.81%, P<0.01), and significantly increased in DSCHF-I compared with DSCHF-V (38.47±1.39%, vs. DSCHF-V, P<0.01) (Fig. 1B).

3.3. RT-PCR for eNOS, iNOS, and type I collagen mRNA expression in the LV

The level of eNOS mRNA in the LV was significantly decreased in DSCHF-V compared with DR-C (0.21±0.02 vs. 0.35±0.01, eNOS mRNA/GAPDH mRNA, P<0.01), and significantly increased in DSCHF-I compared with DR-C and DSCHF-V (0.53±0.05 vs. DR-C and DSCHF-V, P<0.01, respectively) (Figs. 2 and 3A). The level of iNOS mRNA was significantly increased in DSCHF-V compared with DR-C (0.48±0.04 vs. 0.04±0.01, iNOS mRNA/GAPDH mRNA, P<0.01), and significantly decreased in DSCHF-I compared with DSCHF-V (0.14±0.02, vs. DSCHF-V, P<0.01) (Figs. 2 and 3B). Type I collagen mRNA was significantly greater in DSCHF-V than in DR-C (0.66±0.07 vs. 0.18±0.03, type I collagen mRNA/GAPDH mRNA, P<0.01), and was significantly less in DSCHF-I than in DSCHF-V (0.36±0.02, vs. DSCHF-V, P<0.01) (Figs. 2 and 3C).

3.4. Western blot analysis

Data are depicted in Figs. 4 and 5. The eNOS protein mass in the LV of DSCHF-V was about half of that of
Fig. 2. Typical gel electrophoresis of RT-PCR of left ventricular eNOS mRNA, iNOS mRNA, type I collagen mRNA, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA. Lane 1 represents Dahl salt-resistant control rats (DR-C), lane 2 failing heart of Dahl salt-sensitive hypertensive rats treated with vehicle (DSCHF-V), and lane 3 failing heart of Dahl salt-sensitive hypertensive rats treated with imidapril (DSCHF-I).

DR-C (320.7 ± 28.9 vs. 651.3 ± 51.6, P < 0.01). However, the eNOS protein mass in the LV of DSCHF-I was about three-fold larger than that of DSCHF-V (971.1 ± 81.8, vs. DSCHF-V, P < 0.01) (Figs. 4 and 5A). The iNOS protein mass of the LV of DSCHF-V was about two-fold larger than that of DR-C (416.7 ± 44.6 vs. 191.2 ± 16.9, P < 0.01). The iNOS protein mass in the LV of DSCHF-I was slightly less than that of DSCHF-V (271.8 ± 24.4, vs. DSCHF-V, P < 0.05) (Figs. 4 and 5B). These results were almost consistent with those obtained by RT-PCR analysis.

3.5. Myocardial remodelling

The wall-to-lumen ratio increased in DSCHF-V compared with DR-C (0.37 ± 0.01 vs. 0.13 ± 0.01, P < 0.01) and significantly decreased by imidapril treatment (0.21 ± 0.01, vs. DSCHF-V, P < 0.01) (Fig. 6A). The degree of perivascular fibrosis was significantly greater in DSCHF-V than in DR-C (0.76 ± 0.03 vs. 0.24 ± 0.02, P < 0.01), and was also significantly decreased by imidapril treatment (0.47 ± 0.02, vs. DSCHF-V, P < 0.01) (Fig. 6B). Compared with DR-C, myocardial fibrosis was significantly greater in DSCHF-V (2.48 ± 0.08 vs. 0.46 ± 0.02, P < 0.01), and was significantly less in DSCHF-I than in DSCHF-V (0.93 ± 0.04, vs. DSCHF-V, P < 0.01) (Fig. 6C).

4. Discussion

In the myocardium of patients with dilated cardiomyopathy-derived chronic heart failure, reduced eNOS activity and increased iNOS activity have been reported [4]. Furthermore, in the myocardium of patients with dilated cardiomyopathy, ischemic heart disease or valvular heart disease-derived chronic heart failure, it has also been shown that iNOS mRNA expression is enhanced [3]. However, iNOS mRNA expression varies among underly-
in vivo during the transition from the compensated left ventricular hypertrophy stage to the failing stage during a specific period [11–14]. In this model, left ventricular eNOS mRNA and protein expression was reduced, while iNOS mRNA and protein expression was increased. In the myocardium of normal rats, eNOS is mainly detected, but iNOS can not be detected and be induced by endotoxin shock [35]. In isolated myocardial cells, it has also been reported that iNOS is induced by cytokine stimuli [36]. It is speculated that iNOS expression induces excessive and continuous release of NO, causing various myocardial disorders. Since administration of NOS inhibitors enhances positive inotropic actions via adrenergic β-receptors in patients with chronic heart failure [37], differing from findings in healthy volunteers, it is suggested that the NO system is involved in cardiac hypofunction in these patients. Therefore, the decreases in left ventricular eNOS
mRNA and protein expression and increases in iNOS mRNA and protein expression observed in our model may be involved in the onset and deterioration of heart failure in this model.

Imidapril treatment improved the decreases in left ventricular eNOS mRNA and protein expression in the DSCHF-V group. The value was increased even when compared to that in the DR-C group. On the other hand, imidapril treatment significantly inhibited enhancement of left ventricular iNOS mRNA and protein expression in the DSCHF-V group. The effects of bradykinin on left ventricular eNOS and iNOS expression are unclear. However, angiotensin II has been reported to enhance cytokine-stimulated iNOS mRNA expression in rat isolated myocardial cells [38]. Therefore, ACE inhibitors may normalize the NO system of the myocardium via inhibitory effects on the renin–angiotensin system at local myocardial sites.

Antihypertensive therapy improves endothelial function in different models of hypertension [39,40], and ACE inhibitors appear to be particularly effective in hypertensive rats [39,40]. It is known that ACE inhibitors reduce the formation of angiotensin II and inhibit the bradykinin breakdown [41] and may have an effect on the suppression of kininase II, which inactivates bradykinin, a stimulator of NO release [42,43]. The increase in bradykinin concentration resulting from inhibition of its breakdown by ACE inhibitor would increase NO via stimulation of the bradykinin B2 receptor subtype and therefore may contribute to the inhibition of vascular and myocardial structural changes [44]. There is some evidence that local tissue renin–angiotensin systems may be involved in myocardial fibrosis. Angiotensin II was found to stimulate cardiac growth [45], and collagen synthesis in cultured vascular smooth muscle cells [46] and to increase fibroblast proliferation and growth of cardiomyocytes [47]. Therefore, angiotensin II may play an important role in cardiac collagen gene expression and may be responsible for myocardial fibrosis. On the other hand, Ishigai et al. [48] demonstrated that bradykinin inhibits the progression of cardiac hypertrophy due to the increase in NO release and that perindopril produces beneficial effects on cardiac hypertrophy by stimulating the bradykinin–NO pathway in rat cultured heart cells. Furthermore, Hartman [49] indicated that the in vivo cardioprotective effect of ramiprilat can be abolished by antagonizing bradykinin receptors or inhibiting NOS, and that the effect is not related to angiotensin II receptor activity. The potential bradykinin-sparing property of ramiprilat may promote increased bradykinin-stimulated NO production leading to cardioprotection. Part of the cardioprotective effects of ramiprilat/bradykinin/NO may occur locally as demonstrated by the in vitro results using isolated cardiomyocytes. Therefore, endogenously bradykinin–NO pathway may participate in the antihypertrophic property of ACE inhibitors in heart failure.

In the present study, total RNA was assayed by RT-PCR with gene-specific primers for eNOS and iNOS. The results of the RT-PCR showed that eNOS was significantly suppressed and the iNOS mRNA level was significantly increased in DSCHF-V compared with DR-C. In addition, eNOS was significantly increased and the iNOS mRNA level was significantly decreased in DSCHF-I compared with DSCHF-V. Furthermore, the monoclonal antibodies used for Western blot analysis detect both the eNOS and iNOS. The results of the Western blot analysis were almost consistent with those obtained by RT-PCR. Therefore, it is important to assess NOS expression at both the mRNA and protein levels to determine the contribution of regulation of the eNOS and iNOS at the level of gene transcription as well as at the level of protein expression.

ACE inhibitors are prescribed as the first-choice drug for chronic heart failure. It has been reported that these agents not only exhibit life-prolonging effects [50,51] but also improve ejection fraction [52] and exercise tolerance [53] when administered for a long period. In this study, imidapril [54,55], of which the efficacy has been demonstrated in various heart failure models, was administered to salt-loaded DS rats for a long period. As a result, this treatment significantly inhibited increased end-diastolic dimension and decreased fractional shortening in the DSCHF-V group. Furthermore, morphologically, this treatment inhibited not only increased heart weight and vascular thickening but also myocardial interstitial fibrosis, perivascular fibrosis and type I collagen mRNA expression. Several mechanisms may be involved in the beneficial effects of imidapril on cardiac structure and function in failing heart of salt-loaded DS rats. The first, the mechanism by which subdepressor dose of imidapril improves heart failure should involve inhibition of the renin–angiotensin system, independent of systolic blood pressure. The second, it is generally known that ACE inhibitors inhibit degradation of bradykinin. Bradykinin has been reported to improve cardiac function [56] and inhibit fibrosis [57]. Therefore, pathological and functional improvement via bradykinin cannot be denied, but the association of changes in myocardial eNOS and iNOS mRNA and protein with bradykinin should be further examined. In any case, improvement in cardiac function by imidapril and the inhibitory effects of imidapril on remodelling noted in the salt-loaded DS heart failure model we used may have been related to inhibition of the renin–angiotensin system at local myocardial sites and/or inhibitory effects on bradykinin degradation.

In conclusion, subdepressor dose of imidapril may ameliorate the endothelial damage not only by the inhibiting production of angiotensin II but also by the promoting eNOS and inhibiting iNOS mRNA and protein expression in the LV, and this increased eNOS mRNA and protein level may have a role in the improvement of congestive heart failure and myocardial remodelling.
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References

[34] Balligand J, Ungureanu-Longoi D, Simmons WW et al. Cytokine-


[44] Erdös EG. Angiotensin converting enzyme and the change in our concept through the years. Hypertension 1990;26:363–370.


