Temocapril prevents transition to diastolic heart failure in rats even if initiated after appearance of LV hypertrophy and diastolic dysfunction

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Abstract

Objective: Congestive heart failure with left ventricular (LV) diastolic dysfunction and preserved systolic function, i.e. diastolic heart failure (DHF), is often observed in hypertensive patients. Although angiotensin converting enzyme (ACE) inhibitors are widely used as antihypertensive therapy, there is a continued controversy about long-term effect of ACE inhibition on diastolic function. The current study was designed to elucidate a therapeutic effect of ACE inhibitor, temocapril, administration initiated after LV hypertrophy (LVH) and diastolic dysfunction are evident.

Methods: Dahl salt sensitive rats fed on 8% NaCl diet from 7 weeks (hypertensive DHF model) were studied at 13 weeks (n=6) or at 19 weeks following chronic administration of a subdepressor dose of temocapril (0.2 mg/kg/day, TEM(1), n=6) or placebo (TEM(-), n=7) from 13 weeks. Results: Compensatory LVH was associated with prolonged time constant of LV relaxation (Tau) at 13 weeks. In TEM(-), progression of LVH and fibrosis and elevation of LV end diastolic pressure were observed at 19 weeks. Administration of temocapril from 13 weeks prevented the further progression of LVH and fibrosis, attenuated increases in myocardial stiffness constant and Tau, and prevented the development of DHF. These effects were accompanied with the attenuation of decreases in sarcoplasmic reticulum calcium(2+)/ATPase 2a and phosphorylated phospholamban and of hypertrophic signalings' upregulation. Conclusions: This study demonstrated that chronic administration of temocapril exerts a therapeutic effect on diastolic dysfunction and prevents the transition to DHF even if initiated after appearance of LVH and diastolic dysfunction.

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Keywords: ACE inhibitors; Heart failure; Hypertension; Hypertrophy; Ventricular function

1. Introduction

Congestive heart failure with left ventricular (LV) diastolic dysfunction and preserved systolic function, i.e. diastolic heart failure (DHF), is often observed in hypertensive patients, and consists of a high proportion of patients with congestive heart failure [1,2]. The rate of hospitalization and the cost of health care associated with DHF rival those associated with systolic heart failure [3]. In general population, the mortality rate among patients with DHF is four times that among those without heart failure [3]. Nevertheless, pathophysiology or therapeutic strategy of DHF has not been established [1,4]. Recently we have demonstrated that activation of renin angiotensin system is associated with the development of DHF in hypertensive heart [5,6]. Impairment of LV diastolic function, and thus, development of DHF was prevented by chronic angiotensin converting enzyme (ACE) inhibition or angiotensin II type 1 receptor blockade initiated at an early stage when LV hypertrophy (LVH) and diastolic dysfunction were not evident [7]. However, there are very little data pertaining to the effects of pharmacological interventions initiated after LVH and diastolic dysfunction are promoted.

The current animal study was designed to elucidate a therapeutic effect of ACE inhibitor, temocapril, administra-
tion in a subdepressor dose initiated after the appearance of LVH and diastolic dysfunction in the DHF model. We previously demonstrated that hypertension gradually develops in Dahl–Iwai salt-sensitive rats fed on 8% NaCl from 7 weeks, followed by compensatory LVH and impaired relaxation at 13 weeks and in turn by the transition to DHF at about 20 weeks [5,6]. Thus, the administration of temocapril was initiated from 13 weeks in this study. As an underlying cardiovascular disease in this model is a hypertensive heart disease, antihypertensive therapy may well provide beneficial effects in this model. However, LV hypertrophy, one of the determinants of prognosis, frequently progresses in hypertensive patients even under an antihypertensive therapy [8,9]. This suggests that antihypertensive therapy is mandatory but not sufficient, and pharmacological intervention is required to provide beneficial effects on ventricular structure and function independent of its hemodynamic effects. Thus, investigation of pharmacological effects of temocapril on LV structure and diastolic function independent of its depressor effect in DHF is likely to provide clinical implications, and a subdepressor dose was used in the current study.

2. Methods

This study conforms with the guiding principles of Osaka University Graduate School of Medicine with regard to animal care, the American Heart Association on research animal use, and the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH Publication No. 85-23, revised 1996).

2.1. Production of the model

Laboratory chow containing 0.3% NaCl was continuously fed to the male Dahl–Iwai salt-sensitive rats (DIS/Eisai, Tokyo, Japan) and they were defined as control group (n=6). Diet was switched to laboratory chow containing 8% NaCl at 7 weeks for the other male rats (n=19). We randomly selected 6 out of 19 rats to study hemodynamic and pathological characteristics at 13 weeks (the 13wH group). Six out of the other 13 rats were selected and given temocapril hydrochloride (0.2 mg/kg per day, courtesy of Sankyo: the TEM(+) group) by gastric gavage from 13 weeks. The scheme of this study protocol is shown in Fig. 1. The dose of temocapril was determined according to the data in a preliminary study (unpublished data). The other 7 rats were given placebo (the TEM(−) group). The diet and tap water were given ad libitum throughout the experiment. Systolic blood pressure was measured at 7, 13, 17 and 19 weeks using the tail cuff system [5].

2.2. Echo and hemodynamic studies

Transthoracic echocardiographic studies were performed at 7, 13, 17 and 19 weeks to determine LV mass, mid-wall fractional shortening and relative wall thickness in a fashion previously described [5,10]. Specifically, rats were anesthetized with intraperitoneal administration of ketamine HCl (50 mg/kg) and xylazine HCl (10 mg/kg), and were held in the half left-lateral position. Rats were allowed to breathe spontaneously during the echo studies. A commercially available echo machine equipped with a 7.5-MHz transducer (SONOS 2000, Hewlett-Packard, Andover, MA, USA) was used to measure LV inner diameter.
and wall thickness at a paper speed of 100 mm/s. End systolic wall stress was calculated at 19 weeks following Douglas's method [11]. Hemodynamic studies were performed at 13 weeks in 13wH group and at 19 weeks in the other three groups. Soon after echo studies, LV catheterization was performed for measurement of LV end-diastolic pressure and time constant of LV relaxation (Tau) using non-zero asymptote method [5,10,12].

2.3. Determination of myocardial stiffness constant (MSC)

Simultaneous recordings of LV pressure and LV M-mode echogram were obtained in all the rats studied, and myocardial stiffness constant (MSC) was obtained following the method of Sugawara and co-workers as previously described [10,13]. Specifically, LV pressure tracing and M-mode echocardiogram were scanned into a computer system (Power Macintosh 7600/120, Apple Computer), and LV pressure, internal diameter, and wall thickness were digitized over a cardiac cycle. LV mean wall stress (s) was calculated using the following equation:

\[ \sigma = \frac{PD}{4H} \]

where \( P \) is LV pressure, \( D \) is LV short axis diameter and \( H \) is wall thickness of the region of interest. Then, the diastolic \( \sigma - \ln(1/H) \) data points were fitted to a single exponential curve with zero asymptote to calculate myocardial stiffness constant:

\[ \sigma = C \exp[K \ln(1/H)] \]

where \( K \) was determined as myocardial stiffness constant. The mean value of myocardial stiffness constant of the septum and the posterior wall was used for statistical analysis.

2.4. Tissue sampling

Echo and hemodynamic studies were followed by adequate anesthesia and the heart and the lung were harvested to weigh the left ventricle and the lung in a previously described fashion [5]. Samples of the left ventricle for the measurement of hydroxyproline content and the amounts of mRNAs and protein were immediately placed in liquid nitrogen and stored at \(-80^\circ \text{C}\) [6].

2.5. Pathological studies

A part of the left ventricle was fixed with a phosphate-buffered 10% formalin solution for a week. The specimens were embedded in paraffin, and 2-μm thick transverse sections of the organs were stained with hematoxylin and eosin for routine histological examination and with Azan Mallory stain to evaluate the degree of fibrosis. The percent area of fibrosis in the left ventricle at the papillary muscle level in the slices stained with Azan Mallory stain at \(\times 100\) magnification was determined by previously described computer analysis method [6]. Hydroxyproline content was measured according to the method of Stegemann and Stalder [6].

2.6. Quantitative reverse-transcriptase polymerase chain reaction analysis

Quantitative reverse-transcriptase polymerase chain reaction analysis was performed using Prism 7700 Sequence Detector (Perkin-Elmer, Foster, CA, USA) as previously described [6]. We measured mRNAs of atrial and brain natriuretic peptides (ANP and BNP, respectively), preproendothelin-1, cardiotoxin-1 (CT-1), transforming growth factor-β1 (TGF-β1), sarcoplasmic reticulum calcium(2+)-ATPase 2a (SERCA2a), phospholamban (PLN), and glyceraldehyde-3-phosphate-dehydrogenase (GAPDH). These sequences of all oligo-nucleotides used as forward primers, reverse primers and detection probes were shown in Table 1. To correct the efficiency of cDNA synthesis, the amounts of mRNAs were divided by the amounts of GAPDH mRNA and then, was normalized to a mean value of age-matched control group, respectively.

2.7. Western blot analysis

Frozen LV tissues (~100 mg) were homogenized in 1.5 ml of ice-cold lysis buffer containing in mM Tris–HCl 50 (pH 7.4), sodium orthovanadate 0.1, sodium fluoride 50, sucrose 150, phenylmethylsulfonyl fluoride 1, benzamidine 1, EDTA 5, EGTA 2. Subsequently, the samples were centrifuged at 10 000 \(\times g\) for 10 min. The pellet was discarded and the protein content of the supernatant was determined using the DC-protein assay (BioRad, Hercules, CA, USA). For detection of SERCA2a, PLN and Ser\(^{16}\)-phosphorylated PLN, lysates were heated for 3 min at 95 \(^\circ\)C. Lysates containing equal amounts of protein (20 μg) were subjected to SDS–PAGE. Proteins were transferred onto a nitrocellulose membrane according to the manufacturer’s protocol. Membranes were blocked with 5% nonfat dry milk 1 h and incubated with antibody to SERCA2a (Affinity Bioreagents, Golden, CO, USA), PLN (generously gifted by Dr Jerrey Wang) and Ser\(^{16}\)-phosphorylated PLN (Upstate Biotechnology, Charlottesville, VA, USA) overnight at 4 \(^\circ\)C. Each membrane was also incubated with matched secondary antibody (horseradish peroxidase conjugated, KPL Laboratories). Protein bands were visualized using by enhanced chemiluminescence (Amersham Bioscience, Tokyo, Japan). The degree of labeling was quantified by a computer program (NIH) and expressed in relative units.

2.8. Statistical analysis

Results are expressed as mean values±S.E.M. Parame-
Table 1
Sequences of all oligonucleotides used as forward primers, reverse primers and detection probes

<table>
<thead>
<tr>
<th>mRNA</th>
<th>Forward primer sequences</th>
<th>bp of amplification</th>
</tr>
</thead>
<tbody>
<tr>
<td>GAPDH</td>
<td>TATCGGAGCCGCTGGTACCA</td>
<td>144</td>
</tr>
<tr>
<td></td>
<td>TTACTGCGGGCTGGAACC</td>
<td></td>
</tr>
<tr>
<td>ANP</td>
<td>AAATCCCCGTATACAGTGCGG</td>
<td>105</td>
</tr>
<tr>
<td></td>
<td>GGAAGCCTGACCTCATCTTC</td>
<td></td>
</tr>
<tr>
<td>BNP</td>
<td>CCAGAACAATCCACAGATGC</td>
<td>162</td>
</tr>
<tr>
<td></td>
<td>TCGAAGTCTCCTCTGGACATCC</td>
<td></td>
</tr>
<tr>
<td>prepro endothelin-1</td>
<td>TGTCTACTTCTGGCACCCTTG</td>
<td>151</td>
</tr>
<tr>
<td>CT-1</td>
<td>CTACCCCATTTGGAGGCCAA</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>GGAATATGTGCAACAGGG</td>
<td></td>
</tr>
<tr>
<td>TGF-β,</td>
<td>AGAACCCCCATTTGCGTCCC</td>
<td>178</td>
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<td></td>
<td>GAAACGCTTATCGGTCTCC</td>
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<tr>
<td>SERCA2</td>
<td>GAAGGACGTACCTCGACG</td>
<td>131</td>
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<td></td>
<td>CATCCTGACACAGATTCGAC</td>
<td></td>
</tr>
<tr>
<td>PLN</td>
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</tr>
<tr>
<td></td>
<td>AGGTCTGAGGAGGTTCTGAGC</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TCAATACCTTACTGGCTGCGATCATCG</td>
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</tbody>
</table>

3. Results

3.1. Hemodynamics

Systolic blood pressure was elevated in the TEM(−) and the TEM(+) groups to the same degree at 13 and 19 weeks (Table 2). Increases in LV end diastolic pressure and the ratio of lung weight to body weight were observed at 19 weeks in the TEM(−) group, reflecting congestive heart failure, and the increases were prevented by the chronic administration of temocapril (Table 3).

3.2. LV geometrical and functional change

The ratio of LV mass to body weight (LVMI) assessed by echo was greater in the TEM(−) and the TEM(+) groups than in the control group at 13 weeks (Fig. 2). After 13 weeks, LVMI gradually and progressively increased in the TEM(−) group. In contrast, LVMI did not increase thereafter in the TEM(+) group and LVMI was lower in the TEM(+) group than in the TEM(−) group at 19 weeks (Fig. 2). The data of gravitational LV mass and LVMI at 19 weeks support the results obtained by echo (Table 3). LV end-diastolic dimension at 19 weeks was not different among the three groups (Table 2).

LV systolic function as assessed with midwall fractional shortening was not different among the control, TEM(−), and TEM(+) groups throughout the study period (Table 2). Significant changes in LV systolic function were observed in the TEM(−) group at 19 weeks (Table 3).

Table 2
Serial changes in the control group and the rats of group TEM(−) and TEM(+) (n = 6)

<table>
<thead>
<tr>
<th>sBP (mmHg)</th>
<th>BW (g)</th>
<th>MFS (‰)</th>
<th>LVDd (mm)</th>
<th>PWd (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group (n = 6)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7 weeks</td>
<td>116±2</td>
<td>189±12</td>
<td>19±1</td>
<td>6.6±0.2</td>
</tr>
<tr>
<td>13 weeks</td>
<td>143±1</td>
<td>384±7</td>
<td>18±1</td>
<td>9.0±0.1</td>
</tr>
<tr>
<td>19 weeks</td>
<td>135±5</td>
<td>436±10</td>
<td>18±1</td>
<td>9.2±0.2</td>
</tr>
<tr>
<td>TEM(−) group (n = 7)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7 weeks</td>
<td>113±2*</td>
<td>208±6</td>
<td>20±1</td>
<td>6.7±0.2</td>
</tr>
<tr>
<td>13 weeks</td>
<td>224±5*</td>
<td>374±12</td>
<td>18±1</td>
<td>8.3±0.2</td>
</tr>
<tr>
<td>19 weeks</td>
<td>230±2*</td>
<td>352±13*</td>
<td>17±1</td>
<td>9.4±0.2</td>
</tr>
<tr>
<td>TEM(+) group (n = 6)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7 weeks</td>
<td>112±2*</td>
<td>195±6</td>
<td>21±1</td>
<td>6.7±0.1</td>
</tr>
<tr>
<td>13 weeks</td>
<td>220±4*</td>
<td>361±29</td>
<td>20±1</td>
<td>8.3±0.3</td>
</tr>
<tr>
<td>19 weeks</td>
<td>226±4*</td>
<td>371±8*</td>
<td>18±1</td>
<td>8.9±0.1</td>
</tr>
</tbody>
</table>

* Values are expressed as mean±S.E.M. *P <0.05 versus control group.
† P<0.05 versus TEM(−) group. Abbreviations: sBP=systolic blood pressure; BW=body weight; MFS=midwall fractional shortening; LVDd=LV end diastolic dimension; PWd=end diastolic wall thickness of posterior wall.
Table 3
Results of hemodynamics and pathology at 19 weeks

<table>
<thead>
<tr>
<th></th>
<th>Control group</th>
<th>TEM(−) group</th>
<th>TEM(+) group</th>
</tr>
</thead>
<tbody>
<tr>
<td>LVEDP (mmHg)</td>
<td>7±1</td>
<td>15±2*</td>
<td>9±1†</td>
</tr>
<tr>
<td>Lung/body weight (mg/g)</td>
<td>3.3±0.1</td>
<td>7.8±1.4*</td>
<td>4.2±0.3†</td>
</tr>
<tr>
<td>Tau (ms)</td>
<td>23±2</td>
<td>31±1*</td>
<td>26±1*</td>
</tr>
<tr>
<td>ESS (10^3 dynes/cm^2)</td>
<td>89±8</td>
<td>102±14</td>
<td>116±8</td>
</tr>
<tr>
<td>LV mass (g)</td>
<td>0.84±0.01</td>
<td>1.3±0.1*</td>
<td>1.2±0.1*†</td>
</tr>
<tr>
<td>MSC</td>
<td>2.0±0.1</td>
<td>4.3±0.6*</td>
<td>3.6±0.3*†</td>
</tr>
<tr>
<td>LVMi (mg/g)</td>
<td>2.0±0.1</td>
<td>3.9±0.1*</td>
<td>3.1±0.2*†</td>
</tr>
<tr>
<td>Area of fibrosis (%)</td>
<td>2.5±0.1</td>
<td>7.3±0.4*</td>
<td>4.2±0.8*†</td>
</tr>
<tr>
<td>Pro-OH (μmol/g)</td>
<td>2.3±0.3</td>
<td>3.6±0.3*</td>
<td>2.7±0.3†</td>
</tr>
</tbody>
</table>

Values are expressed as mean±S.E.M. * P<0.05 versus control group, † P<0.05 versus TEM(−) group. Abbreviations: Systolic BP=systolic blood pressure; LVEDP=L V end-diastolic pressure; lung/body weight=lung weight corrected to body weight; Tau=time constant of LV relaxation; ESS=end systolic wall stress; MSC=myocardial stiffness constant; LVMI=L V mass corrected to body weight; Pro-OH=hydroxyproline content in LV tissue.

2). Tau was prolonged in both hypertensive groups at 19 weeks, but the prolongation was smaller in the TEM(+) group as compared with the TEM(−) group (Table 3). End systolic wall stress was not different among the three groups at 19 weeks (Table 3). MSC was higher in both the TEM(−) and TEM(+) groups than in the control group but the increase was smaller in the TEM(+) group than in the TEM(−) group (Table 3).

3.3. LV fibrosis

Hydroxyproline content and percent area of fibrosis in the left ventricle were significantly higher in the TEM(−) group than in the control group (Table 3), and histological observation revealed perivascular and interstitial fibrosis particularly in the subendocardial portion in the TEM(−) group. Reduction in hydroxyproline content and percent area of fibrosis is noted in the TEM(+) group (Table 3).

3.4. mRNA levels of hypertrophic and fibrotic stimulating signals

The mRNA levels of ANP and BNP were higher in the TEM(−) group than in the control group. The administration of temocapril significantly attenuated their gene expression (Fig. 2). The levels of prepro endothelin-1 and TGF-β1 mRNA were higher in the TEM(−) group than in the control group. Treatment with temocapril reduced the increases (Fig. 3). The expression of CT-1 mRNA was lower in the TEM(−) group compared to the control group and its expression was upregulated by administration of temocapril (Fig. 3).

3.5. mRNA and protein levels of Ca²⁺ cycling proteins

Decreases in SERCA2a mRNA and protein levels were observed in the TEM(−) group but not in the TEM(+) group (Fig. 4). Neither mRNA nor protein level of PLN
was different among the three groups (Fig. 5). However, phosphorylation level of PLN decreased in the TEM(−) group compared to the control group and this decrease was prevented by temocapril (Fig. 5).

3.6. Comparison of the data between before and after ACE inhibition

In order to study therapeutic effects of temocapril, the data of the TEM(+) group at 19 weeks were compared with those of the 13wH group. While gravitational LVMI (3.1±0.1 mg/g) and hydroxyproline content (3.0±0.2 μmol/g) were not different between these groups, relative wall thickness was smaller in the TEM(+) group than in the 13wH group (51±5 vs. 41±2%, P<0.05), indicating that chronic administration of temocapril attenuated concentric geometry without changes in LV mass or fibrosis. Tau was shortened by temocapril from 13 to 19 weeks (31±1 vs. 26±1 ms, P<0.05).

4. Discussion

Long-term administration of temocapril from the compensatory hypertrophic stage prevented the decreases in SERCA2a and phosphorylated PLN and improved LV relaxation in hypertensive DHF model using Dahl salt-sensitive rats. This treatment also prevented the further...
progression of LVH and fibrosis, leading to attenuation of myocardial stiffening. Thus, long-term administration of temocapril, even if initiated after LVH and impaired LV relaxation are evident, exerts beneficial effects on LV diastolic function in hypertensive hearts. The benefit was brought about independently of its antihypertensive effect and was enough to prevent the transition to DHF.

4.1. Therapeutic effect of temocapril after compensatory hypertrophic stage on LV relaxation

Effect of acute ACE inhibition on LV relaxation abnormality was reported in patients with severe LVH and preserved systolic function [14]. However, effect of long-term ACE inhibition is still controversial in patients with LVH and abnormal relaxation [15,16]. Although chronic ACE inhibition prevented the deterioration of LV relaxation or of Ca\(^{2+}\) uptake in previous in vivo studies [7,17,18], the treatment in any of these studies was initiated before the abnormalities were manifest. The current study expanded the previous studies by demonstrating that long-term administration of temocapril attenuated the advanced abnormality of LV relaxation. This result is partly supported by the ex vivo study demonstrating that the slowing of myocyte relaxation in hypertrophied cardiac myocyte was reversed by ACE inhibition [19].

LV relaxation is at least partly regulated by SERCA2a activity [20]. In this study, long-term administration of temocapril prevented the decrease in SERCA2a mRNA and protein levels, which is consistent with the previous studies of other ACE inhibitors [17,21]. Angiotensin II type 1 receptor-mediated signaling pathway activates protein kinase C, and protein kinase C activation decreases SERCA2a mRNA and protein expression [22]. Thus, the improvement of LV relaxation by temocapril may be at least partly explained by its direct inhibition of SERCA2a downregulation through protein kinase C inhibition. Moreover, long-term administration of temocapril attenuated the decrease in phosphorylated PLN protein level without changing the mRNA or protein level of PLN. If important roles of PLN in sarcoplasmic reticulum Ca\(^{2+}\) transport system are considered [23], functional alteration of PLN, even without a change in protein level, may explain temocapril-induced improvement of LV relaxation.

4.2. Effect of temocapril after compensatory hypertrophic stage on LV structure

Long-term administration of temocapril from the compensatory hypertrophic stage restrained the further progression of LVH (Fig. 2) and fibrosis (Table 3). We showed that progressive LVH after the compensatory hypertrophic stage in the DHF model is maladaptive and excessive and is likely induced with LV fibrosis by activation of renin angiotensin system [7]. The excessive LVH and LV fibrosis contribute to myocardial stiffening and are therapeutic targets for hypertensive DHF [10]. Some previous studies reported that ACE inhibition exerts beneficial effects on LVH or fibrosis in animal models with LV hypertrophy independently of its depressor effects [24–27], however, other studies reported a lack of beneficial effects in subdepressor doses in pressure overload models [28–30]. In addition, the animal models used in the previous studies develop systolic heart failure, not DHF. A recent trend is to consider that systolic heart failure and DHF have different pathophysiology and that their therapeutic strategies should be different [31,32]. Thus, the

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**Fig. 5.** (A) The mRNA levels of PLN at 19 weeks in control, TEM(−) and TEM(+) groups. * P<0.05 vs. control group, † P<0.05 vs. TEM(−) group. Values are means±S.E.M. (B) Representative Western blot analysis of PLN and p-PLN and the protein levels of PLN and p-PLN at 19 weeks in control, TEM(−) and TEM(+) groups. * P<0.05 vs. control group, † P<0.05 vs. TEM(−) group. Values are means±S.E.M. Abbreviation: PLN=phospholamban; p-PLN=Ser\(^{16}\) phosphorylated phospholamban.
results of the previous studies cannot be easily extrapolated to DHF. The early initiation of ACE inhibitor or angiotensin II type 1 receptor blocker before appearance of LVH or diastolic dysfunction prevented the excessive LVH, LV fibrosis and myocardial stiffening in the hypertensive DHF model independent of the depressor effects in other previous study and ours [7,33,34]. The current study expanded the previous studies by demonstrating that the similar benefits were obtained by the long-term ACE inhibition with temocapril in the hypertensive DHF model, even if ACE inhibition was initiated after the appearance of LVH and LV relaxation abnormality.

Gene expression of endothelin-1 and TGF-β1, both of which induce myocyte hypertrophy and fibrosis [35–37] was enhanced in the untreated rats and the enhancement was attenuated in the rats treated with temocapril. As angiotensin II enhances their gene expression in vitro [38,39], the beneficial effects of temocapril may be partly provided through the prevention of upregulation of these growth factors. However, it should be noticed that the administration of temocapril after 13 weeks did not regress LVH. This may be because subdepressor dose of temocapril was used and adaptive LVH for consistent pressure overload was necessary to avoid an elevation of wall stress (Table 3). In this study, CT-1 gene expression was downregulated in the untreated rats but was upregulated by temocapril. CT-1 induces myocyte hypertrophy through prolongation of myocyte length [40] in contrast to endothelin-1 and TGF-β1 that increase myocyte width. Thus, geometrical alteration that temocapril induced from 13 to 19 weeks without change in LVMI may be partly explained by the upregulation of CT-1 and the attenuation of upregulation of endothelin-1 and TGF-β1. As CT-1 exerted a cardioprotective effect in vitro and in vivo [41,42], the temocapril-induced upregulation of CT-1 may partly contribute to the beneficial effects of temocapril in this study. However, our observation does not specify whether the changes in the gene expression in the rats treated with temocapril were causative of or secondary to the temocapril-induced prevention of functional and structural deterioration and DHF. To clarify the mechanisms, further studies are necessary.

In summary, long-term administration of temocapril, even if initiated after diastolic dysfunction and LVH are evident, restrains the further progression of LVH and fibrosis, improves LV relaxation abnormality, and attenuates myocardial stiffening in Dahl salt-sensitive rats with hypertension, resulting in the prevention of the transition to DHF. These benefits are associated with the attenuation of decreases in SERCA2a and phosphorylated PLN, of the upregulation of gene expression of endothelin-1 and TGF-β1, and of the downregulation of CT-1 gene expression. Although chronic ACE inhibition is an established therapeutic strategy for patients with systolic heart failure, its effect on development of DHF is still unclear. In our recent study, administration of ACE inhibitor prevents the impairment of LV diastolic function if initiated before LVH or diastolic dysfunction is observed [7]. Taken together with the current results, ACE inhibition exerts therapeutic as well as preventive effects against the development of DHF in hypertensive hearts and may be a key therapeutic regimen for DHF as well as systolic heart failure.

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