A Natural p300-Specific Histone Acetyltransferase Inhibitor, Curcumin, in Addition to Angiotensin-Converting Enzyme Inhibitor, Exerts Beneficial Effects on Left Ventricular Systolic Function After Myocardial Infarction in Rats

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Background: A natural p300-specific histone acetyltransferase (HAT) inhibitor, curcumin, may have therapeutic potential for heart failure. However, it is unclear whether curcumin exhibits beneficial additive or synergistic effects on conventional therapy with angiotensin-converting enzyme inhibitors (ACEIs).

Methods and Results: Rats were subjected to a sham operation or left coronary artery ligation. One week later, 34 rats with a moderate sized myocardial infarction (MI) were randomly assigned to 4 groups: solvents as control (n=8), enalapril (an ACEI, 10mg·kg⁻¹·day⁻¹) alone (n=8), curcumin (50mg·kg⁻¹·day⁻¹) alone (n=9) and enalapril plus curcumin (n=9). Daily oral treatment was repeated and continued for 6 weeks. Echocardiographic data were similar among the 4 groups before treatment. After treatment, left ventricular (LV) fractional shortening (FS) was significantly higher in the enalapril (29.0±1.9%) and curcumin (30.8±1.7%) groups than in the vehicle group (19.7±1.6%). Notably, LVFS further increased in the enalapril/curcumin combination group (34.4±1.8%). Histologically, cardiomyocyte diameter in the non-infarct area was smaller in the enalapril/curcumin combination group than in the enalapril group. Perivascular fibrosis was significantly reduced in the enalapril/curcumin group compared with the curcumin group.

Conclusions: A natural non-toxic dietary compound, curcumin, combined with an ACEI exerts beneficial effects on post-MI LV systolic function in rats.  

Key Words: Angiotensin-converting enzyme inhibitor; Curcumin; Heart failure; Hypertrophy
cyte hypertrophy, initially the expression level of p300 and its HAT activity are enhanced. Activation of p300 induces the acetylation of not only histone but also GATA4, increases its DNA-binding capacity and up-regulates the expression of hypertrophy-responsible genes. On the other hand, in transgenic mice that overexpress intact p300 in the heart, GATA4 acetylation and LV remodeling after myocardial infarction (MI) are augmented. Such augmentation did not occur in mice that overexpress mutant p300 lacking HAT activity. These findings indicate that the p300 HAT activity is a pharmacological target for HF therapy.

A natural compound, curcumin, derived from Curcuma longa, is a HAT inhibitor and has the ability of p300/CBP-specific HAT inhibition and of cell permeation. In a previous study, we reported that curcumin inhibits hypertrophic responses in cultured neonatal rat cardiomyocytes and prevents the deterioration of LV systolic function in 2 different HF rat models, MI and hypertensive heart disease. Others also report that curcumin prevents diabetes-induced cardiac hypertrophy in rats. These observations suggest that p300-specific HAT inhibitor, curcumin, inhibits maladaptive hypertrophy of cardiomyocytes and exhibits beneficial effects in animal models of HF. To apply this therapy to the clinical setting for HF, it must be clarified whether curcumin is beneficial in combination with widely used agents such as ACEIs. Although ACEIs target extracellular molecules, neurohormonal factors activated in HF may not be restricted to the already known ones such as catecholamines, angiotensin II, endothelin-I and aldosterone. Because curcumin targets a common downstream pathway in cardiomyocytes, we hypothesize that the combination of an ACEI and curcumin would more effectively prevent the development of HF than single therapy with either of these agents.

In the present study, we evaluated the beneficial effects of monotherapy with an ACEI or curcumin and of combination therapy with an ACEI and curcumin on the post-MI LV function in a rat model of HF following MI.

### Methods

#### Animals

Male Sprague-Dawley rats were purchased from Japan SLC Inc. All animal experiments conformed to the Guide for the Care and Use of Laboratory Animals by the Institute of Laboratory Animals, Graduate School of Medicine, Kyoto University.

#### MI Model

MI was created in rats weighing 250–290g by ligating the proximal left anterior descending (LAD) coronary artery through a left thoracotomy, as described previously. The same surgical procedure was performed in sham-operated rats in which the LAD coronary artery was not ligated. One week after LAD ligation, blood pressure (BP) was measured in all rats by the tail-cuff method. Cardiac function of all rats were noninvasively evaluated by echocardiography according to methods described previously. In brief, images were recorded using a 10- to 12-MHz phased-array transducer (model 21380A with HP SONOS 5500 imaging system; Agilent Technologies). Diastolic and systolic LV internal dimensions (LVIDD and LVIDS) and LV fractional shortening (LVFS) were measured with M-mode tracings from the short-axis view of the LV at the papillary muscle level. All measurements were performed in a blinded fashion according to the guidelines of the American Society for Echocardiology and averaged over 3 consecutive cardiac cycles. After the physiological studies, surviving rats were euthanased, and their hearts were excised.

#### Treatments

One week after LAD ligation, 34 post-MI rats with an FS <40% were randomly assigned to 4 groups. Group I (n=6) comprised sham-operation rats with vehicle treatment (1% gum arabic) as the control. Groups II–V comprised MI rats with vehicle (n=8), enalapril (10 mg · kg⁻¹ · day⁻¹) (n=8), curcumin (50 mg · kg⁻¹ · day⁻¹) (n=9), or enalapril (10 mg · kg⁻¹ · day⁻¹) and curcumin (50 mg · kg⁻¹ · day⁻¹) combination (n=9)

### Table 1. BW and Hemodynamic Parameters Before Treatment

<table>
<thead>
<tr>
<th>Treatments</th>
<th>n</th>
<th>BW (g)</th>
<th>HR (beats/min)</th>
<th>SBP (mmHg)</th>
<th>DBP (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham control</td>
<td>6</td>
<td>307±7</td>
<td>356±19</td>
<td>135±4.5</td>
<td>90±3.5</td>
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<tr>
<td>MI vehicle</td>
<td>8</td>
<td>295±4</td>
<td>432±15*</td>
<td>114±2.9*</td>
<td>76±3.8*</td>
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<tr>
<td>MI enalapril</td>
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<td>298±9</td>
<td>405±18*</td>
<td>116±3.7*</td>
<td>72±3.6*</td>
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<tr>
<td>MI curcumin</td>
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<td>305±9</td>
<td>406±12*</td>
<td>111±2.6*</td>
<td>82±2.2*</td>
</tr>
<tr>
<td>MI enalapril/curcumin</td>
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<td>317±8</td>
<td>398±14*</td>
<td>113±4.0*</td>
<td>77±3.2*</td>
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</tbody>
</table>

Values are mean±SE. *P<0.05 vs. sham control.

### Table 2. BW and HW, Hemodynamic Parameters and Infarct Size After Treatment

<table>
<thead>
<tr>
<th>Treatments</th>
<th>n</th>
<th>BW (g)</th>
<th>HW (mg)</th>
<th>HW/BW (mg/g)</th>
<th>LVW (mg)</th>
<th>LVW/BW (mg/g)</th>
<th>HR (beats/min)</th>
<th>SBP (mmHg)</th>
<th>DBP (mmHg)</th>
<th>Infarct size (%)</th>
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<tbody>
<tr>
<td>Sham control</td>
<td>6</td>
<td>468±10</td>
<td>1,093±26</td>
<td>2.34±0.03</td>
<td>792±21</td>
<td>1.69±0.04</td>
<td>364±20</td>
<td>125±6</td>
<td>107±5</td>
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<tr>
<td>MI vehicle</td>
<td>8</td>
<td>453±10</td>
<td>1,126±25</td>
<td>2.49±0.05*</td>
<td>837±24</td>
<td>1.85±0.04</td>
<td>352±14</td>
<td>123±4</td>
<td>105±3</td>
<td>16.7±2.1</td>
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<tr>
<td>MI enalapril</td>
<td>8</td>
<td>406±6*</td>
<td>954±21*</td>
<td>2.35±0.05*</td>
<td>692±19*</td>
<td>1.70±0.05*</td>
<td>387±21</td>
<td>101±4*</td>
<td>85±3*</td>
<td>17.1±4.0</td>
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<tr>
<td>MI curcumin</td>
<td>9</td>
<td>437±8</td>
<td>1,099±24</td>
<td>2.51±0.06</td>
<td>788±25</td>
<td>1.80±0.05</td>
<td>380±18</td>
<td>114±2</td>
<td>97±2</td>
<td>17.5±3.1</td>
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<tr>
<td>MI enalapril/curcumin</td>
<td>9</td>
<td>426±9</td>
<td>958±25*</td>
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<td>385±22</td>
<td>105±3*</td>
<td>87±2*</td>
<td>17.2±2.4</td>
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</table>

Values are mean±SE. *P<0.05 vs. sham control; †P<0.05 vs. MI vehicle.

BW, body weight; HW, heart weight; LV, left ventricular; LVW, left ventricular weight. Other abbreviations see in Table 1.
Figure 1. (A) Representative photographs of M-mode images from the vehicle-, enalapril-, curcumin-, and enalapril/curcumin-treated myocardial infarction (MI) rats. (B–D) Results of echocardiographic parameters. Each value is the mean±SEM from 6 rats in the control group, 8 rats in the vehicle and enalapril groups, and 9 rats in the curcumin group and enalapril/curcumin groups. (B) Left ventricular (LV) internal dimension of diastole. (C) LV fractional shortening. (D) Posterior wall thickness.
Figure 2. (A) Representative cross-sectional H&E-stained images of whole hearts from the vehicle-, enalapril-, curcumin-, and enalapril/curcumin-treated myocardial infarction (MI) rats. Scale bar=2mm. (B) Cross-sectional lumen area of the left ventricle (LV). Values are mean±SEM. (C) Average wall thickness in the non-infarct area of the LV. Values are mean±SEM. (D) Representative cross-sectional images of myocardial cells stained with H&E from each rat. Scale bar=10μm. (E) Myocardial cell diameter measured in at least 50 cells in each rat. Values are mean±SEM.
Arts and Crummin Therapy for HF

treatments administered orally once daily for 6 weeks.

**Histological Analysis**
The excised hearts were cut into 2 transverse slices at the mid-level of the papillary muscles. The specimens were fixed in 10% formalin, embedded in paraffin, sliced into 4-μm-thick sections, and stained with H&E and Masson trichrome. Quantitative assessments of the cross-sectional myocardial cell diameter and perivascular and interstitial fibrosis areas were previously described. The scale of the measured intramyocardial coronary artery was more than 50 μm in each rat. Infarct, lumen, and total area of LV and the extent of wall thickness (WT) opposite the infarct area were traced manually on the digital images and automatically measured. The WT in each group was measured at 3 points in the non-MI area of the LV and presented as the average values. The infarct size, expressed as a percentage, was calculated by dividing the infarcted area by the total area of the LV and multiplying by 100.

**Western Blotting**
Nuclear extracts were prepared from rat LV tissue. Western blotting was performed as previously described. We used rabbit anti-GATA4 polyclonal antibody, rabbit anti-p300 polyclonal antibody (Santa Cruz Biotechnology), and mouse anti-GAPDH monoclonal antibody (Molecular Probes; Invitrogen) for Western blotting. The levels of the signals were detected by photographing them using LAS-1000 Plus (FUJIFILM), quantified by densitometry with the use of Multi Gauge V.3.0 (FUJIFILM), and corrected by using GAPDH.

**Dual Luciferase Assays**
Primary neonatal rat cardiomyocytes were prepared as described previously. The plasmid construct, pANF-luc, has been described previously. Cardiomyocytes were co-transfected with the indicated amounts of DNA using Lipofectamine Plus (Invitrogen) and pretreated with enalapril or curcumin for 2 h. The activities of firefly and sea pansy luciferase were measured in the same cell lysate using the Dual-Luciferase® Reporter Assay System (Promega). The relative promoter activities were calculated as the ratio of firefly to sea pansy luciferase.

**Statistical Analysis**
The results are presented as the mean±SE. Statistical comparisons were performed using ANOVA with Fisher’s test. P<0.05 was taken to indicate significance.

**Results**

**BP and Ventricular Weights**
Although body weight (BW) was comparable at 1 week after coronary artery ligation in all 5 groups, heart rates in the 4 groups with MI were significantly higher than in the sham

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**Figure 3.** (A) Representative images of Masson trichrome-stained sections of the left ventricle (LV) from rats of each group. Scale bar=50 μm. (B) Areas of perivascular fibrosis in the LV were measured for at least 5 intramyocardial coronary arteries with a lumen size >50 μm. Values are mean±SEM. (C) Areas of interstitial fibrosis in the LV were measured. Values are mean±SEM. MI, myocardial infarction.
Systolic and diastolic BP in the 4 groups with MI were significantly lower than in the sham control group (Table 1). As shown in Table 2, there were no significant differences in infarct size among the 4 MI groups at 7 weeks after coronary artery ligation. After treatments, BW in the enalapril group was lower than that in the MI vehicle group (P<0.05). Compared with the MI vehicle group, enalapril and the combination of enalapril and curcumin significantly reduced heart weight (HW) and LV weight (LVW) (P<0.05). HW/BW and LVW/BW in the enalapril and enalapril/curcumin combination groups were also significantly lower than those in the MI vehicle group (P<0.05). In contrast, curcumin did not affect HW or LVW. Systolic and diastolic BP in the 2 groups with enalapril were lowered by its antihypertensive effect. However, curcumin treatment did not affect systolic and diastolic BP (Table 2).

**Echocardiographic Analysis**

There were no differences among the 4 groups with MI with respect to any parameters examined, including BW, BP, WT, LV dimensions and LVFS at baseline and at 1 week after coronary artery ligation (data not shown). Representative M-mode images from vehicle- and enalapril/curcumin-treated MI rats at 6 weeks after the treatments are shown in Figure 1A.
significantly increased LVIDD (P<0.05) and posterior WT (P<0.01), and significantly decreased LVFS by 19.7% (P<0.01). LVIDD in each MI group was comparable (Figure 1B). LV systolic function, represented by LVFS, was significantly higher in the enalapril and curcumin groups than in the vehicle group (each P<0.01). Moreover, the combination of enalapril and curcumin further improved LV systolic function compared with vehicle (P<0.001), enalapril alone (P<0.05) and curcumin alone (P<0.05) groups (Figure 1C). Posterior WT was significantly thinner in the enalapril group than in the vehicle group (P<0.01). Moreover, posterior wall thickening in the curcumin or enalapril/curcumin combination groups was more effectively repressed than in the enalapril-treated group (Figure 1D).

**Myocardial Cell Analysis**

Representative whole images of the transversely sectioned LV in each group are shown in Figure 2A. The same configurations of the heart as shown in echocardiography could be confirmed. The enalapril/curcumin combination treatment reduced the lumen area of the LV and average WT in the non-MI area compared with the vehicle (Figures 2B, C). Images of myocardial cells in each group are shown in Figure 2D. MI increased the cross-sectional myocardial cell diameter and myofibril organization (P<0.01) (Figure 2E, lanes 1 and 2). These increases were significantly inhibited by each treatment (P<0.01) (lanes 2–5). Moreover, the combination of enalapril and curcumin significantly inhibited the enlargement of the myocardial cell size compared with enalapril alone (P<0.05) (lanes 3 and 5).

**Perivascular Fibrosis**

Images of perivascular fibrosis in the non-MI area of each group are shown in Figure 3A. The areas of measured perivascular fibrosis indicated that they notably expanded after MI (P<0.01) (Figure 3B, lanes 1 and 2). The area of perivascular fibrosis became significantly smaller with each treatment (P<0.01) (lanes 2–5). Moreover, the combination of enalapril and curcumin further prevented expansion of perivascular fibrosis compared with curcumin alone (P<0.05) (lanes 4 and 5). However, the area of interstitial fibrosis was comparable among the MI groups (Figure 3C).

**Expression Levels of p300 and GATA4**

To investigate whether combination therapy with enalapril and curcumin affects the expression levels of p300 and GATA4 in the rat heart, we performed Western blotting using nuclear extracts from LV tissue. The p300 and GATA4 expressions are shown in Figure 4A, and quantified in Figures 4B, C. The expression of p300 was increased at 7 weeks after MI compared with the sham-operation. Enalapril treatment and the combination treatment reduced the expression of p300 compared with vehicle treatment. However, curcumin treatment did not affect these changes. The ratio of GATA4/GAPDH was comparable among each group.

**ANF Promoter Activity**

To investigate whether the combination of enalapril and curcumin affects the phenylephrine-induced ANF promoter activity, we performed a reporter assay using neonatal rat cardiomyocytes treated with enalapril and curcumin. The results of ANF promoter activity are shown in Figure 4D. Treatment with curcumin inhibited phenylephrine-induced activity in a dose-dependent manner. However, treatment with enalapril did not alter activation or affect the inhibition of phenylephrine-induced ANF activation by curcumin.

**Discussion**

Salient findings of the present study are that (1) curcumin in addition to enalapril further improved LV systolic function in MI rats, and (2) the combination of enalapril and curcumin further decreased myocardial cell size in the non-MI area compared with enalapril alone. Traditional therapies for chronic HF (eg, ACEI, angiotensin-receptor blocker (ARB), β-blocker and spironolactone, which target extracellular molecules) ameliorate the systolic dysfunction and LV remodeling. However, the mortality and morbidity of HF is still considerably high and it is necessary to further develop the treatment of HF by discovering more effective agents than the existing ones. Accumulating evidence suggests that combination therapy is an acceptable and useful therapeutic approach. The RESOLVD study demonstrated that the combination of enalapril and candesartan was more effective in the prevention of LV remodeling than single therapy with either drug.31 However, combining captopril with valsartan increased the rate of adverse events, such as hypotension and hyperkalemia, without improving survival.32 The contradiction may be related to the similar mechanisms of action of ACEI and ARB on LV remodeling and HF. Therefore, to establish drastic HF therapy, innovative therapy with a different mechanism from that of conventional agents is required.

In particular, this study showed that curcumin exerts beneficial effects on LV systolic function post-MI, in addition to enalapril. However, neither curcumin nor enalapril affected MI size. We started the treatments at 1 week after coronary ligation when MI had been completely established. It has been demonstrated that chronic captopril therapy significantly improves LV systolic function, but does not affect infarct size.33 Our data are compatible with that previous report. Histologically, single therapy with either curcumin or enalapril decreased cardiomyocyte diameter. Curcumin in addition to enalapril further decreased cardiomyocyte diameter. The combination of enalapril and curcumin inhibited the development of LV remodeling after MI. It is well-known that angiotensin II plays a critical role in the development of myocardial fibrosis during pressure overload-induced cardiac hypertrophy.34,35 The combination of enalapril and curcumin reduced perivascular fibrosis, but did not significantly alter interstitial fibrosis. In the present study, the extent of interstitial fibrosis in the non-infarct area was minimal (<1%). Therefore, further studies are needed regarding the effect of curcumin on fibroblasts as well as cardiomyocytes.

In the rat heart, enalapril treatment and the combination treatment with enalapril and curcumin repressed MI-induced p300 expression. In cultured cardiomyocytes, the combination of enalapril and curcumin had no additive effect on ANF promoter activity. Notably, enalapril alone was not sufficient to inhibit phenylephrine-induced ANF activity. This finding may be explained by the fact that the systemic renin–angiotensin system does not exist in vitro. Although genes for angiotensin I and ACE are expressed in the heart, the role of such a local renin-angiotensin system in cultured cardiomyocytes may be minimal during hypertrophy because of the short duration of phenylephrine stimulation. Although ACEI targets extracellular neurohumoral factors, curcumin targets a downstream pathway within cardiomyocytes. These findings suggest differences in the functional mechanisms of enalapril and curcumin. Although the relationship between hypertrophic signals and p300 HAT activity is notable, the
advanced regulation mechanism of p300 is still unclear. Further examinations are needed regarding the regulation of p300 during hypertrophic responses.

It is necessary to investigate whether curcumin exerts additive effects when combined with other conventional drugs, such as ARB and β-blockers, for chronic HF. It has been reported that enalapril prevents the development of HF without affecting BP after MI. The aim of this study was to investigate whether curcumin has an additional effect on chronic HF under the conditions in which enalapril is effective. The dose of 10 mg·kg⁻¹·day⁻¹ of enalapril was relatively high. Indeed, enalapril significantly lowered both the systolic and diastolic BP. However, we demonstrated that curcumin possessed beneficial effects in addition to 10 mg·kg⁻¹·day⁻¹ of enalapril. As curcumin alone was sufficient to exert beneficial effects, it is highly likely that additional effects from curcumin occur even at a dosage of less than 10 mg·kg⁻¹·day⁻¹ of enalapril. Therefore, to apply curcumin therapy in the clinical setting, optimum dose adjustment among curcumin and other drugs such as ACEI and ARB may be important to maximize the beneficial effects of combination therapy for HF.

Curcumin has several attractive properties and possesses diverse pharmacologic effects, including anti-inflammatory, antioxidant, antiproliferative, and antiangiogenic activities. Anand et al. reported that curcumin is safe, even at a high dose (12 g/day) in humans. Dhillon et al. reported that oral curcumin exhibits biological activity in some patients with pancreatic cancer. In spite of its efficacy and safety, the therapeutic efficacy of curcumin has been limited by its poor bioavailability. To develop effective curcumin therapy for HF in humans, it is necessary to improve bioavailability by changes in its formulation, such as the use of nanoparticles and micelles.

In conclusion, we have demonstrated beneficial effects of combination therapy with an ACEI, enalapril, and curcumin on post-MI systolic dysfunction in rats compared with ACEI or curcumin monotherapy. Consequently, the usefulness of curcumin therapy combined with conventional drugs for chronic HF should be clarified in humans.

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References

30. Takaya T, Wada H, Morimoto T, Sunagawa Y, Kawamura T,


