Curcumin Ameliorates Left Ventricular Function in Rabbits with Pressure Overload: Inhibition of the Remodeling of the Left Ventricular Collagen Network Associated with Suppression of Myocardial Tumor Necrosis Factor-α and Matrix Metalloproteinase-2 Expression

Qing-Hai YAO, Dong-Qi WANG, Chang-Cong CUI,* Zu-Yi YUAN, Shao-Bo CHEN, Xiao-Wei YAO, Jun-Kui WANG, and Jiang-Fang LIAN

Department of Cardiology, The First Hospital of Medical School of Xi’an Jiaotong University; Xi’an, China.
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Objective: Curcumin is a wide-spectrum cellular protector with antiinflammatory, antioxidant, and antifibrotic effects. This study was conducted to investigate its effects on myocardial collagen remodeling in pressure overloaded rabbits. Methods and Results: Pressure overloaded rabbits were established by partial abdominal aorta ligation. The rabbits were divided into the sham-operation group, vehicle group and curcumin group. Curcumin was administered orally at a dose of 100 mg/kg·d in 10 ml of 2.5% polyethylene glycol solution and the other 2 groups were given the same dose of polyethylene glycol solution. Compared with the vehicle group, left ventricular function in the curcumin group was significantly ameliorated, as indicated by decreased left ventricular end-diastolic pressure, left ventricle weight to body weight ratio, and the left ventricular posterior wall thickness. The collagen volume fraction in the curcumin group was also reduced. Myocardial tumor necrosis factor (TNF-α and matrix metalloproteinase (MMP)-2 expression were significantly overexpressed in the vehicle group and markedly suppressed in the curcumin group at both the 4th and 8th weeks. At the end of the 8th week, the ejection fraction in the curcumin group was increased compared with that in the vehicle group. Conclusion: Curcumin improved left ventricular function in pressure overloaded rabbits. This might be due to inhibition of collagen remodeling associated with suppression of myocardial expression of tumor necrosis factor-α, and matrix metalloproteinase-2.

Key words curcumin; collagen network; tumor necrosis factor-α; matrix metalloproteinase-2

Ventricular remodeling is a milestone in the progression of congestive heart failure (CHF), which is characterized by cardiomyocyte hypertrophy, apoptosis, and remodeling of the extracellular matrix (ECM). During cardiac hypertrophy transition to heart failure, the composition and types of collagen undergo complex alterations due to neurohormonal disturbance and local cytokine release. CHF is a process of systemic inflammation with overexpression of local inflammatory cytokines including tumor necrosis factor (TNF)-α, interleukin-1 (IL-1) β and matrix metalloproteinases (MMPs). Those inflammatory cytokines may play a crucial role in the development of heart failure.11

Recent clinical trials and experimental studies have provided evidence to suggest that increased cardiac TNF-α expression contributes to the progress of left ventricle (LV) dysfunction.2,3) Experimental studies showed that increased TNF-α levels could cause LV remodeling and promote the deterioration of LV function.4,5) MMPs constitute a family of zinc-dependent enzymes that are responsible for ECM degradation in either a physiological or pathological process.6) Myocardial ECM remodeling regulated by MMPs is implicated in the progression of heart failure. Recent reports have shown TNF-α contributes to the process of myocardial remodeling in evolving heart failure through the local induction of specific MMPs.5-8) Modification of the expression of TNF-α and MMPs may serve as potential therapeutic targets in the treatment of heart failure.

Curcumin is a polyphenol contained in the rhizome of the plant Curcuma longa Linn. Previous studies demonstrated that curcumin is an effective cell protector with antiinflammatory, antioxidant and antifibrotic effects. It can inhibit the expression of a sequence of inflammatory cytokines such as TNF-α, IL-1, or IL-89) and MMP-3, MMP-13,10) MMP-211) in the kidney or derma. Curcumin is a potential cleanser of oxidized free radicals (OFRs) and this effect is much stronger than that of carotene or vitamin E12) due to its ability to suppress the synthesis of xanthine oxidase and lower its activity,13) but to enhance the activity of the superoxide dismutase (SOD) enzyme.14) Curcumin can retard the fibrotic process in the lung,15) liver, or kidney.16) It is proposed that this effect is associated with down-regulation of TNF-α expression, reducing lipid peroxidization and blocking the transmission of apoptotic signals.16) Curcumin suppresses renal parenchymal cell apoptosis in mice in which the lateral ureter was ligated to inhibit activation of nuclear transcription factor (NF)-κB and down-regulate the expression of Fas-ligand.

In the present study, we tested the hypothesis that curcumin could improve cardiac function and counteract myocardial collagen remodeling of failing hearts. We also explored the influence of curcumin on the expression of TNF-α and MMP-2 in the LV myocardium of pressure overloaded rabbits.

MATERIALS AND METHODS

Animal Models Twelve-week-old New Zealand White rabbits were provided by the Animal Center of Xi’an Jiaotong University, Xi’an, China. Pressure overload was established by partial abdominal aorta ligation according to Cuttilletta et al. method with slight modification.17) All procedures were in accordance with the Guide for the Care and Use of

* To whom correspondence should be addressed. e-mail: CZCui@263.net © 2004 Pharmaceutical Society of Japan
Laboratory Animals published by the US Animal Care Institute. Rabbits were anesthetized with pentobarbital sodium 30 mg/kg. The abdominal aorta was exposed and paralleled with a 5-F sheath. Both the sheath and the abdominal aorta were tied with a silk thread at a proximal site 5—10 mm from the left renal artery, and then the sheath was quickly withdrawn. This resulted in a limited (approximately 50%) stenosis of the abdominal aorta. The celiac organs were replaced and sutured to the muscles or skin. Animals in the sham-operated group underwent the same process except for aorta ligation.

**Treatment Protocol**  Rabbits with pressure overload were divided randomly into the curcumin group (curcumin, n=20) and control group (vehicle, n=20). Curcumin was dissolved in 2.5% polyethylene glycol solution, and 2% curcumin solution (SanAisi, Shanghai, China) was administered at a dose of 100 mg/kg·d for 4 or 8 weeks by oral gavage from 24 h after surgery. The control group and the sham-operated group were given the the same dose of vehicle (2.5% polyethylene glycol solution).

**Measurement of Cardiac Function** The ejection fraction (EF) and left ventricular posterior wall thickness (PWT) of all animals were measured while conscious with Doppler echocardiography (HP 2500 SONOS, U.S.A.).

Hemodynamic parameters and left ventricular weight to body weight ratios (LVW/BW) were measured at the end of the 4th or 8th week. In anesthetized rabbits with the right carotid artery exposed the left ventricular end-systolic pressure (LVESP) and left ventricular end-diastolic pressure (LVEDP) were recorded using a PowerLab pressure-monitoring system (AD Instrument, Australia). The hearts were excised and rinsed with cold saline. The atrium and right ventricle were removed. The left ventricles were weighed for calculating the LVW/BW. Part of myocardial tissue was removed from the ventricular free wall and fixed in 10% neutral buffered formalin for 48 h, and then embedded in paraffin.

**Measurement of Collagen Content** Tissue sections were stained with van Giesson (VG) solution composed of Diamant fuchsin and saturated bitter acid in the proportion of 1:2. Tissue sections including the nonvessel collagen volume fraction (CVF-NV) and vessel collagen volume fraction (CVF-V). Immunohistochemistry To detect the expression of TNF-α and MMP-2, we used the streptavidin peroxidase (S-P) immunohistochemical staining technique. After deparaffinization, the sections were incubated with 0.03% H2O2–PBS for 20 min to block endogeneous peroxidase activity. After routine antigen treatment with microwave, the sections were blocked with normal goat serum for 12 min, then successively incubated with primary antibody (1:100 goat-and-rabbit polyclonal TNF-α and MMP-2 affinity purified antibody (Boster Company, Wuhan, China) for 24 h (4 °C), secondary antibody (Elivision Kit, Maixing Company, Fuzhou, China) for 60 min, and diaminobenzidine [DAB kit (20×), Zhong-Shan Company, Beijing, China] for 10 min. Finally, all sections were dehydrated in absolute ethanol, cleared in xylene, and sealed with gum. Six visual fields in each section were randomly selected. To analyze the gray values of TNF-α and MMP-2, the Leica Q550CW image system was used and the averages were calculated.

**Statistical Analysis** All values are expressed as mean± standard deviation (S.D.). One-way analysis of variance (ANOVA) followed by Fisher’s protected least significant difference test were performed. A value of p<0.05 was considered statistically significant.

**RESULTS**

**Changes in Cardiac Function and Histopathologic Parameters in Pressure Overloaded Rabbits** As shown in Table 1, Fig. 1 and Fig. 2, compared with the sham-operated group, the LVESP, LVEDP, LVW/BW, PWT, and CVF in the vehicle group were increased significantly at the end of the 4th week (p<0.01); The gray values of TNF-α and MMP-2 were decreased significantly (p<0.01). At the end of the 8th week, LVESP, LVEDP, LVW/BW, and CVF in the vehicle group were much higher than those in the sham-operated group (p<0.01), while the gray values of TNF-α and MMP-2 (p<0.01), PWT, and EF (p<0.05) were lower than those in the sham-operated group. LVW/BW, CVF, LVESP, and LVEDP measured at the end of the 8th week were much higher than those at the 4th week in the vehicle group (the first three were p<0.01, the latter was p<0.05). PWT, EF, and gray values of TNF-α and MMP-2 were lower than those at the 4th week (the first two were p<0.05, the latter two were p<0.01). There was no significant difference at the 4th or 8th week in the sham-operation group (p>0.05).

**Curcumin Ameliorated Left Ventricular Function in Rabbits with Pressure Overload** (Table 1) There was no

<table>
<thead>
<tr>
<th>4 Week</th>
<th>8 Week</th>
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<tbody>
<tr>
<td>Sham</td>
<td>Vehicle</td>
</tr>
<tr>
<td>LVESP (mmHg)</td>
<td>120±11.30</td>
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<tr>
<td>LVEDP (mmHg)</td>
<td>0.49±0.29</td>
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<tr>
<td>PWT (mm)</td>
<td>3.10±0.40</td>
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<tr>
<td>EF</td>
<td>0.82±0.07</td>
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<tr>
<td>LVW/BW</td>
<td>1.47±0.09</td>
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Comparison between curcumin and vehicle, *p<0.05, **p<0.01; between vehicle and sham, *p<0.05, **p<0.01; comparison between parameters of 4th and 8th weeks, 1p<0.05, 2p<0.01.
difference in LVEDP and EF between the curcumin and vehicle groups at the end of the 4th week ($p < 0.05$) (Table 1). At the end of the 8th week, LVEDP in the curcumin group was depressed and EF was raised in the vehicle group ($p < 0.05$). There was no significant difference in LVESP between the curcumin and vehicle group at either time point ($p > 0.05$).

**Curcumin Inhibited the Remodeling of the Left Ventricular Collagen Network in Rabbits with Pressure Overload (Table 1, Figs. 1, 2)**

LV/BW and CVF (including CVF and CVF-NV) in the curcumin group were lower than those in the sham group throughout the entire period ($p < 0.01$). Curcumin remarkably decreased the CVF-NV and CVF-V at both time points (at the 4th week, $p < 0.05$; at the 8th week, $p < 0.01$). CVF-NV was represented by the percentage of collagen area to total area in one visual field not containing small vessels, while CVF-V was represented by the percentage of collagen area to total area containing small vessels.

**Curcumin Suppressed the Myocardial Expression of TNF-α and MMP-2 in Rabbits with Pressure Overload**

The mean gray values of TNF-α and MMP-2 in the vehicle group were significantly lower throughout the entire period compared with those in the sham group ($p < 0.01$). Curcumin raised the values of TNF-α and MMP-2 at both the 4th and 8th weeks ($p < 0.01$). Gray values were in inverse proportion with actual TNF-α and MMP-2 expression levels. Means of each group are shown.

**DISCUSSION**

The present findings clearly demonstrate that curcumin effectively ameliorates LV function of failing hearts in rabbits with pressure overload. The present results also show that curcumin inhibits the myocardial collagen deposition associated with suppression of the myocardial expression of TNF-α and MMP-2 in rabbits with pressure overload.

According to our results, rabbits with partial abdominal aorta ligation underwent the transition from cardiac hypertrophy to heart failure. LVESP, LVDSP, PWT, and LV/BW were all markedly elevated in the vehicle group compared with the sham-operation group at the 4th week. This indicated typical LV hypertrophy. At the end of the 8th week, LVESP and LVDSP increased progressively, while PWT and EF decreased, meaning that heart failure was induced by pressure overload. Although pressure overload initially involved the left ventricle, its hemodynamic and humoral consequences exerted a graded effect on the right ventricle. Some rabbits in the vehicle group also showed clinical signs of total heart failure, such as tachypnea, ascites, and gallop rhythm. Myocardial collagens ensured the structural integrity of adjoining cardiomyocytes and provided the means by which myocyte shortening was translated into overall LV pump function. Our findings hinted that CVF-V and CVF-NV increased progressively in the vehicle group, which might result in less compliance of the LV wall and impaired diastolic function. In the vehicle group, CVF-NV was higher than CVF-V at the same time point, implying that collagen...
deposition was initiated around the small vessels distributed in the myocardium. Many trials and studies have been reported to support the potential importance of TNF-α in the pathogenesis of this disease process. In the present study, we demonstrated that the expression of TNF-α was elevated continuously in the myocardium of pressure overloaded rabbits. Increased TNF-α has been shown to cause significant changes in the ECM. MMP-2 is also called gelatin A which can degrade a wide spectrum of interstitials such as I, IV, V, and VII collagen and elastic fibers. Curcumin is a pharmacologically safe compound with anti-inflammatory, anti fibrotic, and free radical-scavenger properties. It has been documented that curcumin suppresses TNF-α and MMPs in organs such as the kidney or derma. Curcumin can also attenuate fibrosis of the liver, lung, and kidney. In this study we therefore investigated the effects of curcumin on collagen deposition and TNF-α and MMP-2 expression in the LV in a pressure overloaded model.

Curcumin Ameliorates the LV Function in Rabbits with Pressure Overload In the present research, curcumin reduced LVEDP at the 8th week. This suggests that an improvement in LV diastolic function occurred, which might result from the increased LV compliance due to curcumin. However, there was no significant change in LVESP at either time point. At the end of the 8th week, EF in the curcumin group was higher than that in the vehicle group, indicating an amelioration of LV systolic function. Tharaux et al. reported that curcumin could inhibit fibrosis of the aorta and renal artery which led to an improvement in vessel elasticity and inactivation of the renin-angiotensin system. This might decrease the blood pressure indirectly. The equilibrium of enhanced cardiac contractive force and decreased blood pressure was postulated to underlie the slight change in LVESP between the sham and vehicle groups.

Curcumin Inhibited the Myocardial Collagen Deposition in Pressure Overloaded Rabbits In our findings, LVW/BW, CVF-V, and CVF-NV in the curcumin group were much lower than those in the vehicle group at both time points. Moreover, histomorphologic results offered evidence that the distribution or alignment of collagen fibers were more regular in the curcumin group, suggesting that curcumin suppressed myocardiac collagen remodeling in pressure overloaded rabbits in our study. Theoretically, an increase in MMP activity would cause a degradation of collagen, but conversely MMP-2 expression increased progressively corresponding to collagen content, which appears paradoxical. However, in the hypertrophic or failing heart, myocardial collagen synthesis exceeds the rate of degradation. Degraded products of collagen fibers may serve as a stimulator conducive to more collagen synthesis. MMPs also contribute to LV dilation by collagen network disruption. This may underlie the mechanism by which curcumin blocked the...
Curcumin Suppressed the Myocardial Expression of TNF-α and MMP-2. We found that curcumin potentially inhibited TNF-α and MMP-2 in hypertrophic or failing hearts in rabbits with pressure overload after treatment with curcumin 100 mg/kg·d for a span of time. The underlying mechanism has not been well defined, but some data have been provided about the influence of curcumin on other diseases. Brennan and O’Neill documented that curcumin could markedly inhibit the activation of NF-κB,20 which is a potential transcription inducer for TNF-α and MMP-2. It is clear that an important determinant of overall transcription is the production of transcriptional factors that bind to the gene. Common promoter-binding regions also exist on MMP genes for transcription factors such as AP-125) or NF-κB.26 Another mechanism is related to the antioxidant effect of curcumin. Mauriz et al. also demonstrated that oxidative stress resulting from hemorrhagic shock led to overexpression of TNF-α and activation of NF-κB, while this phenomenon was blunted by antioxidant glycine.26

In conclusion, curcumin improved the LV function in pressure overloaded rabbits, and this effect may result from the inhibition of myocardial collagen remodeling associated with suppression of overexpression of TNF-α and MMP-2.

REFERENCES