A Novel Drug Delivery System of Oral Curcumin Markedly Improves Efficacy of Treatment for Heart Failure after Myocardial Infarction in Rats

Yoichi Sunagawa,a,b,c Hiromichi Wada,b Hidetoshi Suzuki,c Hiroki Sasaki,c Atsushi Imaizumi,d Hiroyuki Fukuda,d Tadashi Hashimoto,d Yasufumi Katanasak,c Akira Shimatsuc,e Takeshi Kimura,f Hideaki Kakeya,g Masatoshi Fujitaa,a Koji Hasegawab and Tatsuya Morimotoc,e

aHuman Health Sciences, Graduate School of Medicine, Kyoto University; bDepartment of Cardiovascular Medicine, Graduate School of Medicine, Kyoto University; Kyoto 606–8507, Japan; cDivision of Translational Research, Clinical Research Institute, Kyoto Medical Center, National Hospital Organization; dClinical Research Institute, Kyoto Medical Center, National Hospital Organization; Kyoto 612–8555, Japan; eDivision of Molecular Medicine, School of Pharmaceutical Sciences, University of Shizuoka; Shizuoka 422–8526, Japan; fTheravalues Corporation; Tokyo 102–0094, Japan; and gDepartment of System Chemotherapy and Molecular Sciences, Division of Bioinformatics and Chemical Genomics, Graduate School of Pharmaceutical Sciences, Kyoto University; Kyoto 606–8501, Japan.

Received June 6, 2011; accepted November 10, 2011; published online November 14, 2011

Curcumin is an inhibitor of p300 histone acetyltransferase activity, which is associated with the deterioration of heart failure. We reported that native curcumin, at a dosage of 50 mg/kg, prevented deterioration of the systolic function in rat models of heart failure. To achieve more efficient oral pharmacological therapy against heart failure by curcumin, we have developed a novel drug delivery system (DDS) which markedly increases plasma curcumin levels. At the dosage of 0.5 mg/kg, DDS curcumin but not native curcumin restored left ventricular fractional shortening in post-myocardial infarction rats. Thus, our DDS strategy will be applicable to the clinical setting in humans.

Keywords curcumin; drug delivery system; heart failure

Signals through hemodynamic overload finally reach the nuclei of cardiac myocytes and activate hypertrophy-responsive transcriptional factors such as GATA-binding protein 4, myocyte enhancer factor 2 and serum response factor.1,2) Activities of these factors are regulated by histone deacetylases and an intrinsic histone acetyltransferase (HAT), p300.3—5) Curcumin is an inhibitor of p300 HAT activity and widely employed as a healthy food diet. Recently, we have reported that native curcumin, at the dosage of 50 mg/kg, prevents the deterioration of systolic function in rat models of hypertension- or myocardial infarction (MI)-induced heart failure.6,7) However, native curcumin powder is insoluble in water, and the absorption of orally administered curcumin is limited. While curcumin, at 50 mg/kg/d, may be safe in humans,8) the intestinal absorption efficiency at this dosage is inadequate in this form. Since the amount of daily water intake is limited in patients with congestive heart failure, a large volume of water to take medicine is a burden for these patients. Therefore, a more efficient system for heart failure therapy will be desirable in actual clinical practice. We have developed a curcumin drug delivery system (DDS), by which the absorption efficiency is much more favorable than that of native curcumin powder.9) The present study examined a hypothesis that this system is useful for heart failure therapy in post-MI rats.

MATERIALS AND METHODS

Preparation of DDS Curcumin Curcumin powder was extracted from Indian turmeric by using alcohol. DDS of curcumin preparation was previously described.8) Shortly, gum ghatti, mainly consists of polysaccharides, obtained from the exudation of ghatti trees, was dissolved in water to make gum ghatti solution. Curcumin powder was mixed into this solution, and water and glycerin were added to adjust the weight. This mixture was ground by a wet grinding mill (DYNOMILL® KDL, Willy A Bachofen AG, Switzerland), and then, dispersed by a high-pressure homogenizer (Homogenizer 15MR-8TA, APV Gaulin). DDS curcumin consisted of 10 w/w% curcumin, 2% other curcuminoids such as demethoxycurcumin and isodemethoxycurcumin, 46% glycerin, 4% gum ghatti, and 38% water.

Chemicals Curcumin powder, mepronil, β-glucuronidase (from Helix pomatia), distilled water (H2O), acetotoninile (MeCN), methanol (MeOH), formic acid (FA), sodium acetate, and chloroform were purchased from Wako (Osaka, Japan).

Animals Male Sprague-Dawley (SD) rats weighing 250—290 g were purchased from Japan SLC Inc. All animal experiments were conformed to the Guide for the Care and Use of Laboratory Animals by the Institute of Laboratory Animals, Graduate School of Medicine, Kyoto University.

Plasma Concentration of Curcumin Curcumin powder (WAKO) was directly suspended in the 1% acacia gum solution to produce native curcumin solution. DDS curcumin solution containing 10% of curcumin was dispersed in the 1% gum ghatti solution. The final content of curcumin in the solution was 1% in both curcumin powder and DDS curcumin. Rats were randomly assigned to 8 groups, and given curcumin powder or DDS curcumin at a dosage of 0.5, 5, 50 and 300 mg curcumin/kg body weight, respectively. The samples were orally administered to rats by direct stomach intubation using gastric catheters. Two hours after administration, blood was taken from the tail of rats and placed into heparinized tubes. Plasma was immediately prepared by centrifugation at 1000g for 10 min at 4°C and stored at –20°C until use. Each plasma sample was incubated with 0.1 μmol sodium acetate buffer (pH 5.0) containing 1000 U β-glucuronidase at 37°C for 1 h to hy-
drolyze the curcumin conjugates. After extraction with chloroform, the dried extracts were reconstituted in 100 mL of 50% MeCN containing 0.05% and injected into a chromatographic system. Plasma concentrations of curcumin were measured using the HPLC-MS/MS system comprising the Prominence micro-LC system (Shimadzu, Kyoto, Japan) and an API 3200 tandem mass spectrometer (Applied Biosystems, CA, U.S.A.) with (+) electrospray ionization (ESI), as described previously.

**Myocardial Infarction Model and Assessment by Echocardiography** Male Sprague-Dawley rats were orally tracheally intubated after being anesthetized with ethyl ether gas. Anesthesia was maintained during the operation with 1 to 1.5% isoflurane. MI was created in rats 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 that LAD coronary artery was not ligated. One week after the LAD ligation, blood pressures were measured in all rats by the tail-cuff method. Cardiac function of all rats was noninvasively evaluated by echocardiography according to the methods described previously. In brief, echocardiography was performed while the rats were lightly anesthetized with ethyl ether gas, and images were recorded using a 10- to 12-MHz phased-array transducer (model 21380A with HP SONOS 5500 imaging system; Agilent Technologies). Left ventricular end-diastolic diameter (LVEDD), left ventricular posterior wall thickness (LVPWTh), and left ventricular 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.

**Treatments** One week after the LAD ligation or sham operation, five sham-operated rats were assigned to vehicle treatment (1% gum ghatti) as controls. Sixteen post-MI rats with an LVFS of smaller than 40% were randomly assigned to the following 4 treatment groups. Groups I to IV comprised MI rats with the treatments of vehicle (1% acacia gum) (n=3), native curcumin powder (0.5 mg/kg/d) (n=5), vehicle (gum ghatti) (n=3), or DDS curcumin (0.5 mg/kg/d) (n=5). Oral administration in each group was continued daily for 6 weeks.

**Histological Analysis** The excised hearts were cut into 2 transverse slices at the mid-level of papillary muscles, fixed in 10% formalin, and stained with hematoxylin and eosin (H&E) and Masson trichrome. Quantitative assessments of the cross-sectional myocardial cell diameter and perivascular and interstitial fibrosis areas were performed as previously described. The size of the measured intramyocardial coronary artery was more than 50 μm in each rat.

**Statistical Analysis** The results are presented as the mean±S.E. Statistical comparisons were performed using analysis of variance (ANOVA) with Fisher’s test. p<0.05 was taken to indicate significance.

**RESULTS**

**DDS Curcumin Markedly Increases Plasma Levels of Curcumin in Rats** To improve the absorption efficiency of curcumin, we prepared a surfactant-soluble, oral DDS curcumin preparation. Subsequently, a single dose of native curcumin powder or DDS curcumin preparation at 0.5, 5, 50, or 300 mg/kg was orally administered to SD rats, and plasma levels were measured after 2 h, when the levels reached a peak. The plasma levels of curcumin in rats treated with DDS curcumin were approximately 60 times higher compared with those treated with native curcumin powder (Fig. 1A). The plasma levels of native curcumin at 0.5 and 5 mg/kg were not detected. Furthermore, the plasma levels following native curcumin powder administration at 50 mg/kg (10.7±1.7 ng/mL), which was effective for heart failure, were similar to those following DDS curcumin administration at 0.5 mg/kg (5.0±2.4 ng/mL) and 5 mg/kg (37.4±13.2 ng/mL) (Fig. 1B).

**DDS Curcumin Improves LV Systolic Function after MI in Rats** Using a rat MI model, we investigated whether the oral DDS curcumin preparation improves heart failure at a dose much lower than that of native curcumin. Effects of this preparation on post-MI cardiac function were examined by echocardiography. There were no differences in the rat body weight, blood pressure, heart rate, LVEDD, LVPWTh,
or LVFS before drug administration among the 4 MI groups (Table 1A). The LVEDD was significantly larger and the LVFS was significantly smaller in each MI group compared with the sham operation group. Figure 2 shows representative photographs of M-mode images from each group. There were no differences in LVFS between the native curcumin treatment and the vehicle (acacia gum)-treated group (14.1 ± 0.9%) and 13.6 ± 0.7%). There were no significant differences in body weight, systolic and diastolic blood pressures, and heart rates among all groups at 6 weeks after the start of treatments. Among MI groups, the LVPWTh was significantly reduced by the DDS curcumin treatment, but not by the native curcumin treatment. In addition, there were no side effects of the oral DDS curcumin administration based on the results of hematology and histological examination.

**DDS Curcumin Reduces Myocardial Cell Hypertrophy and Perivascular Fibrosis in Post-MI Rats** After physiological examinations, we performed histological analysis. Representative images of myocardial cells in each group are shown in Fig. 3A. At 7 weeks after MI, H&E staining showed that MI increased the myocardial cell diameter and myofibrillar organizations (Fig. 3B, lanes 1, 2, 4). Although there were no differences in myocardial cell diameters between the native curcumin powder (0.5 mg/kg/d)-treated group and vehicle (acacia gum)-treated groups (lanes 2, 3), DDS curcumin (0.5 mg/kg/d) treatment significantly reduced these increases compared with the vehicle (gum ghatti) (lanes 4, 5). The perivascular fibrosis areas were expanded at 7 weeks after MI (Figs. 4A, B, lanes 1, 2, 4). The DDS curcumin (0.5 mg/kg/d)-treated group but not the native curcumin powder (0.5 mg/kg/d)-treated group significantly suppressed MI-induced increases in perivascular fibrosis (Fig. 4B, lanes 3, 5). However, the interstitial fibrosis areas were similar among 4 groups with MI (Fig. 4C).

### Table 1A. Hemodynamic Parameters before Treatment (at 1 Week after MI)

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>BW (g)</th>
<th>SBP (mmHg)</th>
<th>DBP (mmHg)</th>
<th>HR (bpm)</th>
<th>LVEDD (mm)</th>
<th>LVPWTh (mm)</th>
<th>LVFS (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham operation</td>
<td>5</td>
<td>332±2</td>
<td>128±3</td>
<td>85±4</td>
<td>299±18</td>
<td>7.7±0.3</td>
<td>1.1±0.2</td>
<td>57.6±4.5</td>
</tr>
<tr>
<td>MI acacia gum</td>
<td>3</td>
<td>319±5</td>
<td>125±6</td>
<td>74±7</td>
<td>300±7</td>
<td>8.8±0.3*</td>
<td>1.3±0.2</td>
<td>29.6±2.4*</td>
</tr>
<tr>
<td>MI native curcumin</td>
<td>5</td>
<td>326±9</td>
<td>109±9</td>
<td>78±4</td>
<td>332±36</td>
<td>8.6±0.4*</td>
<td>1.2±0.1</td>
<td>29.9±2.6*</td>
</tr>
<tr>
<td>MI gum ghatti</td>
<td>3</td>
<td>317±3</td>
<td>131±19</td>
<td>79±4</td>
<td>338±12</td>
<td>8.9±0.5*</td>
<td>1.3±0.2</td>
<td>29.2±1.6*</td>
</tr>
<tr>
<td>MI DDS curcumin</td>
<td>5</td>
<td>318±2</td>
<td>116±5</td>
<td>81±5</td>
<td>309±28</td>
<td>8.8±0.2*</td>
<td>1.2±0.1</td>
<td>30.7±1.9*</td>
</tr>
</tbody>
</table>

### Table 1B. Hemodynamic Parameters after Treatment (at 7 Week after MI)

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>BW (g)</th>
<th>SBP (mmHg)</th>
<th>DBP (mmHg)</th>
<th>HR (bpm)</th>
<th>LVEDD (mm)</th>
<th>LVPWTh (mm)</th>
<th>LVFS (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham operation</td>
<td>5</td>
<td>479±6</td>
<td>123±1</td>
<td>91±3</td>
<td>329±13</td>
<td>8.8±0.3</td>
<td>1.3±0.1</td>
<td>51.4±2.3</td>
</tr>
<tr>
<td>MI acacia gum</td>
<td>3</td>
<td>460±6</td>
<td>133±5</td>
<td>96±9</td>
<td>301±13</td>
<td>10.2±0.1*</td>
<td>2.4±0.3*</td>
<td>13.6±0.4*</td>
</tr>
<tr>
<td>MI native curcumin</td>
<td>5</td>
<td>460±12</td>
<td>114±3</td>
<td>92±3</td>
<td>316±44</td>
<td>9.9±0.4*</td>
<td>2.3±0.2*</td>
<td>14.1±0.9*</td>
</tr>
<tr>
<td>MI gum ghatti</td>
<td>3</td>
<td>467±12</td>
<td>116±5</td>
<td>84±6</td>
<td>298±6</td>
<td>10.6±0.7*</td>
<td>2.6±0.2*</td>
<td>13.8±0.7*</td>
</tr>
<tr>
<td>MI DDS curcumin</td>
<td>5</td>
<td>469±12</td>
<td>133±8</td>
<td>93±2</td>
<td>303±15</td>
<td>10.1±0.3*</td>
<td>1.3±0.2*</td>
<td>25.9±2.7*</td>
</tr>
</tbody>
</table>

Values are mean±S.E. BW=body weight, SBP=systolic blood pressure, DBP=diastolic blood pressure, HR=heart rate, LVEDD=left ventricular end-diastolic diameter, LVPWTh=left ventricular posterior wall thickness, LVFS=left ventricular fractional shortening. *p<0.05 vs. Sham operation. †p<0.005 vs. MI gum ghatti.
DISCUSSION

We have developed DDS curcumin, of which the absorption efficiency was much more adequate than that of native curcumin powder. The present study has demonstrated that an extremely low dose of DDS curcumin improves LV systolic function in post-MI rats, suggesting the clinical usefulness of oral curcumin preparation by this system for heart failure treatment.

Curcumin is a polyphenol that is responsible for the yellow color of the spice turmeric and is commonly used for its perceived health benefits, especially in traditional Indian medicine. It has been demonstrated that curcumin has several biological and pharmacological properties such as anti-cancer, anti-inflammatory, and anti-oxidant effects. As a result, curcumin may be used as a potential treatment of various human disorders. Curcumin has the possibility of delaying the progression of Alzheimer’s diseases by reducing amyloid deposition, it reduces clinical relapse in patients with inflammatory bowel disease, and inhibits the progression of several cancers, including pancreas and colon cancer. In addition, our previous report demonstrated that oral curcumin treatment prevents the deterioration of LV systolic function in two different rat heart failure models, myocardial infarction and hypertensive heart disease. Moreover, we showed the beneficial effects of the combination therapy with an angiotensin converting enzyme (ACE) inhibitor, enalapril, and curcumin on the post-MI systolic dysfunction. Our findings indicated that curcumin treatment might be a candidate for an effective agent for chronic heart failure therapy.

Although curcumin is known to be safe even at a high dose (12 g/d) in humans, its therapeutic efficiency is limited due to its poor bioavailability. The major reasons for the low bioavailability of curcumin are its poor water solubility and absorption. Moreover, absorbed curcumin is rapidly metabolized in the liver and systematically eliminated. Since curcumin in aqueous buffer (pH 5.0) is 11 ng/mL, the oral bioavailability is just about 1%. To improve the bioavailability, many studies have been performed mainly based on changes in drug formation, using a suitable solvent, nanoparticles, and micelles.

Recently, some investigators proposed several delivery approaches to enhance the bioavailability and biological effects of curcumin. Gao et al. showed that the area under curve (AUC) of the plasma concentration curve in the formation of
Curcumin nanosuspension was 3.8-fold greater than that of native curcumin in rabbits. In the present study, we generated a highly absorptive curcumin dispersed with colloidal nanoparticles which is dissolved in gum ghatti solution. The mean particle size of curcumin of our system was 0.19 μm. Oral DDS curcumin yielded higher C_{max} and shorter T_{max} values, as well as a higher AUC in the plasma concentration curve of curcumin (at least 27-fold higher) compared with oral native curcumin powder. These results indicated that the gastrointestinal absorption of curcumin was markedly enhanced. In this study, the plasma concentration of curcumin in the low dosage of oral DDS curcumin (0.5 mg/kg) was 5.0±2.4 ng/mL, while it was not detected in the same dosage of native curcumin. Furthermore, the plasma level following native curcumin powder administration at 50 mg/kg (10.7±1.7 ng/mL), which was effective for rat heart failure, was similar to that of DDS curcumin at 0.5 mg/kg. These results suggest that DDS curcumin could be an effective therapy for heart failure such as MI or hypertensive heart diseases. Subsequently, we evaluated the effect of DDS curcumin on rat post-MI heart failure. Oral DDS curcumin (0.5 mg/kg/d) treatment significantly improved the systolic function and reduced the posterior wall thickness in MI rats, although the same dosage of oral native curcumin (0.5 mg/kg/d) could not exhibit therapeutic effects. Furthermore, DDS curcumin treatment also significantly suppressed the increases of the myocardial cell diameter and perivascular fibrosis. Importantly, histological and hematological analyses indicated that there were no side effects of the oral DDS curcumin administration (data not shown). These results indicate that DDS curcumin is therapeutically effective for heart failure in a small amount compared with native curcumin.

It is a burden to patients with heart failure to take a large amount of medicine. Since DDS curcumin is effective in a small amount, such patients can easily take this medicine every day for a long period. Clinical studies on heart failure patients using DDS curcumin are needed in the near future.

Acknowledgments We thank N. Okamura, N. Chiba, and S. Ura for their excellent technical assistance. This work was supported in part by a Grant-in-Aid to K. Hasegawa and T. Morimoto for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan from the SENSIN Medical Research Foundation, and for Research on Publicly Essential Drugs and Medical Devices from the Japan Health Sciences Foundation. Y. Sunagawa is a research fellow of the Japan Society for the Promotion of Science.
REFERENCES


