Mangiferin diminishes chronic fructose overconsumption-induced cardiac fibrosis in spontaneous hypertension rats

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Abstract: Cardiac fibrosis is the most important cause of left ventricular diastolic dysfunction and heart failure in patients with hypertension. Mangiferin is one of the prominent components found in many kinds of fruits and traditional herbal medicines. This study investigated the effect of mangiferin on cardiac fibrosis. Male spontaneous hypertension rats (SHRs) consumed liquid fructose in their drinking water over 7 weeks, and mangiferin (15 mg/kg, once daily by oral gavage) was co-administered. Cardiac interstitial fibrosis was determined using Masson’s trichrome staining. The indexes of lipid and glucose homeostasis were determined enzymatically. Gene expression was analyzed by real-time PCR. The results showed that mangiferin diminished long-term fructose feeding-induced excessive cardiac interstitial collagen accumulation. However, mangiferin treatment showed little effect on blood pressure, cardiac hypertrophy and systemic metabolic parameters. Gene expression profile revealed that mangiferin suppressed fructose-stimulated cardiac mRNA overexpression of CD68, monocyte chemoattractant protein-1 and transforming growth factor-beta-1, while was without effect on urokinase plasminogen activator and plasminogen activator inhibitor-1 mRNA. Thus, the present results suggest that mangiferin treatment diminishes long-term fructose overconsumption-induced cardiac fibrosis by inhibiting overexpression of macrophage-associated proinflammatory cytokines in SHRs. The anti-inflammatory effect of mangiferin in the heart may occur independently of its systemic metabolic effects.

Keywords: Mangiferin, heart, fibrosis, anti-inflammation

Introduction

It has been recognized that hypertension damages organs seriously, such as heart, brain, kidney and so on, in which the heart disease is the leading cause of death in hypertension patients. Hypertension for long is prone to left ventricular diastolic dysfunction, and cardiac fibrosis is the most important cause of left ventricular diastolic dysfunction and heart failure in patients with hypertension.

Mangiferin, a xanthone glucoside, is found in many kinds of fruits and traditional herbal medicines, such as mangoes (Mangifera indica) and Salacia species [1], and Anemarrhena asphodeloides [2]. Mangiferin and the associated herbs have been demonstrated to display a wide range of pharmacological activity in anti-inflammatory, antioxidant, and anti-diabetes [3-8]. Our previous studies have shown that treatment with mangiferin ameliorates fatty liver and renal interstitial fibrosis in fructose-fed spontaneously hypertensive rats [9, 10]. It is still unknown whether mangiferin is also effective on cardiac remodeling including fibrosis.

Strong evidence suggests that chronically high consumption of fructose results in the metabolic syndrome and other metabolic abnormalities, including obesity, insulin resistance, fatty liver and dyslipidemia [11]. Although the direct cardiomyopathic consequences of chronic high fructose intake are not yet mechanistically well defined, a number of experimental studies have demonstrated that ventricular dysfunction is
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associated with elevated dietary fructose [12-14]. In the present study, we examined the effects of mangiferin on chronic excessive liquid fructose consumption-induced cardiac fibrosis in spontaneously hypertensive rats (SHRs) and further investigated the underlying mechanisms of action.

Materials and methods

Animals, diet and experimental protocol

All experimental procedures were carried out in accordance with the internationally accepted principles for laboratory animal use and care, and approved by the Animal Ethics Committee, Chongqing Medical University, China.

Male SHRs/Nrcl weighing 230-250 g were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd, China, and the standard diet was supplied by the Laboratory Animal Center, Chongqing Medical University, China. Rats were housed under specific pathogen-free conditions in a temperature and humidity controlled facility (21±1°C, 55±5% relative humidity) with a 12-h light/dark cycle. Animals were allowed free access to water and the standard diet for at least 1 week prior to starting the experiments. We have recently demonstrated that treatment of fructose-fed SHR with mangiferin at 15 mg/kg, but not 5 mg/kg, showed a significant anti-steatotic and anti-renal fibrosis effect [9, 10]. Therefore, the dosage of 15 mg/kg was chosen in the present study.

Eighteen SHRs were divided into 3 groups (n = 6 per group): (1) water control, free access to water; (2) fructose control, free access to 10% fructose solution (w/v, preparation every day); (3) fructose+mangiferin 15 mg/kg group. Animals in mangiferin-treated groups were administered mangiferin 15 mg/kg (≥98%, Sigma-Aldrich, Chongqing, China, suspended in 5% Gum Arabic solution, gavage once daily) for 7 weeks. The rats in the corresponding water- and fructose-control groups received vehicle (5% Gum Arabic) alone. Systolic blood pressure (SBP) was measured in conscious rats by a tail-cuff method (MK-2000ST; Muromachi Kikai Co Ltd, Tokyo, Japan) prior to and 7 weeks after the experiment commenced. At least six readings were taken for each measurement. All rats had free access to the standard chow. To avoid stress and maintain accurate monitoring of fructose intake, only 2 rats were housed in a cage at any given time. The consumed chow and fructose solution were measured per 2 rats daily and the intake of fructose was calculated.

At the end of week 6, blood samples were collected by retroorbital venous puncture under ether anesthesia after overnight fast. Here, the plasma concentrations of glucose and total cholesterol (kit from Kexin Institute of Biotechnology, Shanghai, China), insulin (kit from Morinaga Biochemical Industries, Tokyo, Japan), triglyceride; and nonesterified fatty acids (NEFA) (Triglyceride-E kit and NEFA-C kit; Wako, Osaka, Japan) were determined using enzymatic methods or by enzyme linked immunosorbent assay. Overnight-fasted rats were sacrificed under anesthesia at the end of week 7. The heart was dissected out, the weights of the whole heart and left ventricle were measured. Segments of the left ventricle were snap frozen in liquid nitrogen and stored at -80°C for subsequent analysis of cardiac lipid droplet accumulation and determination of triglyceride and total cholesterol contents and gene expression. One part of the left ventricle was fixed with 4% paraformaldehyde-PBS fixative at normal temperature for analysis of cardiac interstitial fibrosis.

Histomorphological examination

A portion of left ventricle was fixed with 4% paraformaldehyde-PBS and embedded in paraffin. To determine the degree of collagen fiber accumulation, three-micron thick sections were cut and stained with Masson’s trichrome. Cardiac interstitial collagen accumulation was assessed by two different researchers in a blinded manner under microscopy (BX-53, Olympus Corporation, Tokyo, Japan). Forty fields in different sections were randomly selected, and Masson’s trichrome-stained area (blue) and total tissue area were determined, their ratio was

| Table 1. Primer sequences for real-time PCR assays (5’ to 3’) |
|-----------------|-----------------|
| Gene            | Forward primers  | Reverse primers |
| β-actin         | ACGGTCAAGGTATCAATATCG | GCCATAGAGGTCTTTACGGATG |
| CD68            | TGGGGCTCTTGGGAAACTACA | CTTTGTTTTTGGTCGGTTCA |
| MCP-1           | CGGTCTCCATTGCTTCTCCTG | GTCGCGCTGACCCCTTTATG |
| TGF-β           | GATCGCTCAGCAAGTCGAGG | CAGGTGTGGACCCCTTTCAG |
| uPA             | GCTTCGGACAAAGAGATGGCCA | GCCATAGTAGTGAGGCTGTTCG |
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Figure 1. Cardiac interstitial fibrosis (A) and representative images showing cardiac interstitial fibrosis (blue) in left ventricle (Masson's trichrome staining, (B-D) scale bars: 50 μm). The fructose controls (Fru) had free access to 10% fructose in their drinking water over 7 weeks, and the consumption of fructose in the mangiferin (MA, 15 mg/kg)-treated (by gavage daily) rats was adjusted to match that of the fructose controls. The water controls (Con) had free access to a tap water. Data are represented as mean±SEM (n = 6 each group). vs. control *P<0.05.

Determination of cardiac triglyceride and total cholesterol contents

Cardiac triglyceride and total cholesterol contents were determined as described previously [9]. Briefly, 100 mg of tissue was homogenized and extracted with 2 ml of isopropanol. After centrifugation (3000 rpm, 10 min at 4°C), the triglyceride and total cholesterol contents in supernatants were determined enzymatically (Wako, Osaka, Japan).

Real-time PCR

Real-time PCR was performed as described previously [9, 15, 16]. Total RNA was isolated from cardiac muscle of individual rats using TRIzol (Takara, Dalian, China). cDNA was synthesized using M-MLV RTase cDNA Synthesis Kit (Takara, Dalian, China) according to the manufacturer's instructions. Real-time PCR was performed with the CFX 96 Real Time PCR Detection System (Biorad Laboratories Inc, Hercules, CA, USA) using the SYBR® Premix Ex Taq™ II (Takara, Dalian, China). The sequences of primers (Takara, Dalian, China) are shown in Table 1. The gene expression from each sample was analyzed in duplicates and normalized against the internal control gene β-actin. Levels
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Data analysis

All results are expressed as mean±SEM. Data were analyzed by ANOVA using StatView, and followed by Student-Newman-Keuls testing to locate the differences between groups. P<0.05 was considered to be statistically significant.

Results

Effect on cardiac interstitial collagen accumulation in rats

Masson’s trichrome staining showed that fructose feeding increased cardiac interstitial collagen deposit (Figure 1C), compared to the control fed water (Figure 1B). The ratio of the Masson’s trichrome-stained area to total tissue area in fructose control group was increased significantly (Figure 1A). Supplementing with mangiferin significantly inhibited this increase (Figure 1A and 1D, P<0.05).

Effects on blood pressure, heart mass and cardiac lipid droplet accumulation in SHRs

Consistent with the well-known characteristic, SHRs were hypertensive (SBP ≈ 190 mmHg). There was no difference in SBP between groups in the beginning and at the end point of experiment (Table 2). SHRs also showed increase in heart weight and left ventricular weight, compared to the corresponding Wistar-Kyoto rats (1.125 g vs 1.109 g and 0.909 g vs 0.787 g, respectively, our unpublished data). SHRs had low cardiac triglyceride (Figure 2C) and total cholesterol (Figure 2D) contents, and lipid droplet (Figure 2E). Both fructose feeding and treatment with mangiferin showed no significant effect on blood pressure, cardiac heart weight, left ventricular weight, cardiac triglyceride and total cholesterol contents, and droplet deposit (Table 2; Figure 2A-E).

Effects on metabolism-associated variables in SHRs

Fructose control and fructose mangiferin groups consumed similar amount of fructose intake during seven weeks (Table 2). Intake of fructose decreased chow intake in the same manner in both fructose control and fructose mangiferin groups (Table 2). There was no significant difference in body weight among water control, fructose control and mangiferin groups. Fructose feeding significantly increased fasting plasma concentrations of triglyceride, NEFA and insulin, while was with little effect on fasting plasma glucose and total cholesterol concentrations (Table 2). Treatment with mangiferin did not affect all of these parameters in fructose-fed SHRs (Table 2).

Cardiac gene expression profile

Compared to water control, fructose feeding increased cardiac expression of mRNAs corresponding to MCP-1 (Figure 3A), CD68 (Figure 3B), TGF-β1 (Figure 3C), and PAI-1 (Figure 3D), whereas it did not affect cardiac uPA mRNA level (Figure 3E). However, the ratio of uPA to PAI-1 mRNA was decreased (Figure 3F). Treatment with mangiferin significantly suppressed overexpression of MCP-1, CD36 and TGF-β1 (Figure 3A-C), while it did not affect PAI-1, uPA and the ratio of uPA to PAI-1 mRNA in fructose-fed SHRs (Figure 3D-F).

Discussion

Cardiac fibrosis is probably one of the major biological determinants of fatal episodes including congestive heart failure, severe arrhythmias, and sudden cardiac death. Therefore, prevention and/or reversal of fibrosis may improve organ function and survival. In our pre-

Table 2. Effects of mangiferin on SBP, fructose and chow intakes, and blood biochemical parameters in SHRs

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Water control</th>
<th>Fructose control</th>
<th>Fructose mangiferin</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBP before (mmHg)</td>
<td>189.5±2.0</td>
<td>193.2±2.5</td>
<td>187.6±3.2</td>
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<tr>
<td>SBP after (mmHg)</td>
<td>204.0±2.8</td>
<td>207.3±4.2</td>
<td>198.7±3.7</td>
</tr>
<tr>
<td>Fructose intake (g/7 w)</td>
<td>—</td>
<td>1024±68*</td>
<td>1074±67</td>
</tr>
<tr>
<td>Chow intake (g/7 w)</td>
<td>308.2±4.8</td>
<td>310.4±6.4</td>
<td>314.0±5.9</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>85.2±3.5</td>
<td>83.7±4.6</td>
<td>83.0±4.8</td>
</tr>
<tr>
<td>Plasma glucose (mg/dl)</td>
<td>43.0±3.5</td>
<td>87.7±5.3*</td>
<td>82.3±6.8</td>
</tr>
<tr>
<td>Plasma triglyceride (mg/dl)</td>
<td>75.7±4.6</td>
<td>77.7±4.9</td>
<td>75.3±5.2</td>
</tr>
<tr>
<td>Plasma NEFA (mEq/L)</td>
<td>1.09±0.04</td>
<td>1.56±0.05*</td>
<td>1.49±0.06</td>
</tr>
<tr>
<td>Plasma insulin (ng/ml)</td>
<td>0.62±0.005</td>
<td>0.83±0.01*</td>
<td>0.82±0.01</td>
</tr>
</tbody>
</table>

Data are represented as mean±SEM (n = 6 each group), *P<0.05 vs control.
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Figure 2. Heart weight (A), left ventricular weight (B), cardiac triglyceride (C) and total cholesterol contents (D), representative images showing cardiac lipid droplet accumulation (red) in left ventricle (Oil Red O staining, (E-G) scale bars: 50 μm). The fructose controls (Fru) had free access to 10% fructose in their drinking water over 7 weeks, and the consumption of fructose in the mangiferin (MA 15 mg/kg)-treated (by gavage daily) rats was adjusted to match that of the fructose controls. The water controls (Con) had free access to a tap water. Data are represented as mean±SEM (n = 6 each group), vs. control *P<0.05.

Previous findings, mangiferin has been demonstrated to alleviate renal interstitial fibrosis induced by high fructose in SHRs [10]. The present results clearly demonstrated that treatment with mangiferin decreased fructose feeding-induced cardiac interstitial collagen accumulation in SHRs. However, mangiferin had minimal effect on fructose and chow intakes, blood pressure, cardiac mass and lipid contents, fasting plasma concentrations of glucose, insulin, total cholesterol, triglyceride and NEFA. Thus, these results suggest that mangiferin treatment diminishes fructose-induced cardiac fibrosis independently of its metabolic and other cardiac effects in SHRs.

It is generally considered that there are two types of cardiac fibrosis: reparative and reactive fibrosis. Reparative fibrosis appears to be a reaction to the loss of myocardial material (due to necrosis or apoptosis, after myocardial ischemia or senescence) and is primarily interstitial in location. In contrast, reactive fibrosis is observed in the absence of cell loss and appears to be a reaction to inflammation [17]. The fibroblasts are co-localized with inflammatory cells in myocardial fibrosis areas of SHRs, and the area of fibrosis is related to the density of infiltrating macrophages [18, 19]. Mononuclear cells were the main infiltrating inflammatory cells in the heart of hypertensive rats. MCP-1 is a monocyte/macrophage chemokine, which may activate and induce the monocyte chemotaxis, exude inflammatory cells and enhance macrophage infiltration [20-22]. Therefore, inhibition of monocyte chemokine and its induced macrophage infiltration may be a new target for the treatment of cardiac fibrosis in hypertension. The present study showed that cardiac mRNA expression of MCP-1 and CD68, a molecular marker of macrophage infiltration, was upregulated by long-term high fructose-feeding. Treatment with mangiferin suppressed fructose-stimulated overexpression of MCP-1 and
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CD68. These results suggest that amelioration of cardiac fibrosis by mangiferin is associated with inhibition of macrophage-associated inflammatory factors in fructose-fed SHRs.

Macrophages secrete a variety of cytokines, including TGF-β1 [23, 24]. TGF-β1 is a potent stimulator of extracellular matrix protein synthesis (e.g., collagen and fibronectin) [25]. It induces differentiation of fibroblasts to myofibroblasts, which has a higher activity for collagen production than fibroblasts [26]. TGF-β1 has been proved to increase collagen biosynthesis in various systems by autoinduction or paracrine regulation [27], both in vivo and in vitro [28, 29], and to promote the pathological of fibrosis [30, 31]. Mangiferin has been demonstrated to have various anti-inflammatory effects [32-35]. In the present study, mangiferin treatment reversed fructose-induced increase in cardiac TGF-β1 expression. These results further confirm the association of mangiferin treatment-elicited amelioration of cardiac fibrosis with its anti-inflammatory effect in fructose-feeding SHRs.

PAI-1 has a number of important roles in pathophysiological processes, such as inhibition of fibrinolysis, regulation of extracellular matrix turnover and activation of proenzymes and latent growth factors that promote tissue fibrosis and sclerosis. The altered uPA to PAI-1 ratio reflects

Figure 3. Cardiac mRNA expression of monocyte chemoattractant protein (MCP)-1 (A), CD68 (B), transforming growth factor-beta (TGF-β)-1(C), plasminogen activator inhibitor (PAI)-1 (D), urokinase plasminogen activator (uPA ) (E), and the ratio of uPA to PAI-1 mRNA (F). The fructose controls (Fru) had free access to 10% fructose in their drinking water over 7 weeks, and the consumption of fructose in the mangiferin (MA 15 mg/kg)-treated (by gavage daily) rats was adjusted to match that of the fructose controls. The water controls (Con) had free access to a tap water. Data are means±SEM (n = 6 each group), vs. control *P<0.05.
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a change from a profibrinolytic to an antifibrinolytic state [36]. It is demonstrated that insulin resistance increases PAI-1 in the heart [37]. In the present study, long-term consumption of fructose leads to insulin resistance in SHRs. Consistently, cardiac PAI-1 mRNA expression was also increased, while the ratio of uPA to PAI-1 mRNA was downregulated. Treatment with mangiferin showed no effect on cardiac PAI-1 mRNA expression and the ratio of uPA to PAI-1 mRNA in fructose-fed rats.

Lipid accumulation in nonadipose tissues has been increasingly recognized to contribute to organ injury through a process termed lipotoxicity. There is substantial evidence that excess renal lipids cause injury under conditions including obesity, diabetes, aging, and myocardial ischemia-reperfusion [38]. Lipotoxic cellular dysfunction and injury occur through several mechanisms such as release of proinflammatory and profibrotic factors [38]. Fructose consumption may induce excessive lipid accumulation in liver [9, 16]. In the present study, however, Seven-week fructose feeding did not alter lipid contents in SHRs. Mangiferin also did not affect myocardial lipid contents in fructose-fed rats. Thus, it is unlikely that MA treatment ameliorates fructose-induced cardiac fibrosis via modification of lipid metabolism in rats.

In conclusion, our results demonstrate that mangiferin treatment ameliorates chronic fructose overconsumption-induced cardiac fibrosis in SHRs by inhibiting macrophage-associated inflammatory response. The anti-inflammatory effect of mangiferin in the heart may occur independently of its systemic metabolic effects. Our findings provide a potential therapeutic target of mangiferin treatment for cardiac fibrosis.

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Disclosure of conflict of interest

None.

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