Original Article

Chaihu Shugan Powder attenuates Liver Qi stagnation type of metabolic syndrome in spontaneously hypertensive rats by upregulating liver X receptor-alpha

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Abstract: The objective of this study was to establish a MetS of Liver Qi Stagnation rat model and to observe the effects of Chaihu Shugan Powder on the main components of MetS and the expression of LXR mRNA in the liver tissues. A MetS of Liver Qi Stagnation model was established in spontaneously hypertensive rats (SHR) by combination of a high-fat and high-sucrose diet with Bondage of Modern Stimulation Method for 12 weeks. Forty male SHR were randomly divided into control group and MetS of Liver Qi Stagnation model group. Rats in the control group were further randomized into normal diet control (NC) and CSP-intervention (NI) group. Rats in the MetS model group were further randomized into high-fat diet control (FC) and CSP-intervention (FI) group. CSP (24.8 g/kg/d) was administered by gavage for 8 weeks. Biochemical and physiological parameters and mRNA expression of liver X receptor (LXR) α and β were determined. Following CSP treatment, rats in the FI group had an obvious reduction in total cholesterol, low density lipoprotein, and HOMA-IR than the FC group (all P<0.05). Rats in the FI group exhibited markedly increases in liver LXRα mRNA expression than the FC group (P<0.05). CSP attenuates the main components of MetS of Liver Qi Stagnation by up-regulating LXRα mRNA expression in the SHR.

Keywords: Chaihu Shugan Powder, metabolic syndrome, Liver Qi stagnation, liver X receptor-α

Introduction

Metabolic Syndrome (MetS) is a morbid condition including central obesity, dyslipidemia, hypertension, hyperglycemia, insulin resistance, proinflammatory and prothrombotic state [1]. MetS represents an emerging global public health burden [2]. Worldwide prevalence of MetS depends on age, gender, race, ethnicity and its definition [3, 4]. In China, the pooled prevalence of MetS was approximately 24.5% among individuals aged 15 years and older [5]. MetS is an important contributor to cardiovascular morbidity and mortality [6] and type 2 diabetes [7]. Current therapeutic strategies in MetS are mainly dietary approach, regular physical activity, and use of pharmacological measures to control individual components [8]. Therefore, developing new therapies for MetS are urgently required due to its deleterious effects.

Traditional Chinese Medicine (TCM) has been shown to possess beneficial effects against MetS [9, 10]. Chaihu Shugan Powder (CCP) is a TCM prescription originating from Qing-Dynasty. Ingredients of CSS include Bupleurum Chinense, Pericarpium Citri Reticulatae, Radix Paeoniae Alba, Rhizome of Chuanxiong, Radix Glycyrrhizae, Fructus Aurantii, and Rhizoma Cyperi. CCP has been used to treatment for the liver-depression syndrome [11-13]. Moreover, administration of CCP has achieved beneficial effects in regulation of glucose or lipid homeostasis and insulin resistance [14-17]. However, the exact mechanisms of its action remain poorly understood.

Liver X receptors (LXRs) are ligand-activated transcription factors belonging to the family of nuclear receptors [18]. LXRs are commonly classified as LXRα and LXRβ. LXRα expression is predominant in metabolic tissues, while LXRβ is ubiquitously expressed [19]. LXRα plays an important role in the genetic susceptibility to MetS [20]. LXRs have been increasingly recognized as pivotal regulators of cholesterol homeostasis, lipid and glucose metabolism.
CSP treatment for MetS

[21, 22]. Therefore, regulation of LXRs may represent a promising therapeutic target for MetS.

No previous study has observed the effect of CCP on MetS. The objective of this study was to establish a MetS of Liver Qi Stagnation rat model and to observe the effects of CCP on the main components of MetS and the expression of LXR mRNA in the liver tissues.

Materials and methods

Animals and diets

All experiments were performed in accordance with institutional guidelines for the Care and Use of Laboratory Animals and approved by Guangzhou University of Traditional Chinese Medicine. Sixty male specific pathogen-free spontaneously hypertensive rats (SHR) weighing (260.6 ± 3.28 g) were purchased from the Vital River Laboratory Animal Technology Co. Ltd. Beijing (Certificate number: 1140070004936). All rats were maintained at 19-23°C with 50 ± 5% humidity on a 12 h light/dark cycle with free access to drinking water.

Rat feed was obtained from Guangdong Medical Laboratory Animal Center. The normal diet formula was composed of 10% fat, 22% protein, 68% carbohydrates, and 0.5% (g/g) salt, and the high-fat diet formula was composed of 55% normal diet, 12% lard, 5% sucrose, 8% powdered milk, 5% peanut, 10% egg yolk powder, 3% sesame oil, 2% salt, to which was added 15 ng thiamine hydrochloride, 15 ng riboflavin, 10 ng pyridoxine hydrochloride adjoin, 10 ng folic acid, 10 ng vitamin K3, 50 ng Calcium pantothenate, 20 ng niacin, 1 g choline chloride, and 0.5 g inositol per kg. Sucrose drinking water was freshly prepared with analytical grade sugar (Guangdong MiaoBo Biotechnology Co. Ltd.) and ultrapure water at 1:10.

Preparation of CSP

CSP is composed of the following Chinese herbs: Bupleurum Chinense 120 g, Pericarpium Citri Reticulatae 100 g, Radix Paeoniae Alba 100 g, Rhizome of Chuanxiong 100 g, Radix Glycyrrhizae 60 g, Fructus Aurantii 100 g, and Rhizoma Cyperi 100 g. Raw herbs were identified by two senior pharmacists at the Guangdong hospital of TCM pharmacy, decocted in distilled water and condensed to 1.24 g/ml, considered crude drug. After autoclave sterilization, the decocted solutions were kept at 4°C.

The rat model of MetS with Liver Qi stagnation

After 2 weeks, rats were randomized into control and MetS model groups. Rats in the MetS group received the high-fat, high-sucrose diet for 12 weeks, and were placed in stainless steel tubes to restrict activity (Figure 1) for 30 min 4 times per day, during which time feeding was not restricted [23]. The model was considered established in animals exhibiting the following features: unresponsive, slowed or significantly reduced behavior, reduced alertness and response to external aural stimuli, dodged for the external irritability. Sparse, dry, and yellowed fur, narrowed eyes with mucoid discharge, dry tails with brown scales, dry or thin droppings and weight loss. The body weight of rats in the MetS group was over 1.4 standard deviations greater than the normal diet group. Model establishment was also assessed by open-field test performance, using a rectangular open wooden box enclosure with a black wall of 150 (length) × 150 (width) × 75 (height) cm. The floor of the box was divided into 25 10 cm² squares. A single rat was placed in the center of the floor. We counted the number of squares crossed by all four paws of the rats to indicate activity. The number of times each rat rose up on its hind legs was also counted. Each rat was tested once for 3 minutes.
CSP treatment for MetS

Animal grouping and drug administration

Following successful establishment of the MetS Liver Qi stagnation model, 20 rats were further randomized to the high-fat diet control (FC) and CSP-intervention (FI) group. Rats in the control group were randomized into the normal diet control (NC) and CSP-intervention (NI) group. CSP (1 ml/100 g) was administered twice a day by gavage for 8 weeks. Rats in the FC and NC group received normal saline by gavage.

Measurement of physiological parameters

Body weight was determined by electronic weighing scale. Abdominal circumference was determined by measuring tape around the anterior abdomen. Body length was determined by measuring the distance from the head to rump. Systolic blood pressure (SBP) was measured using a noninvasive blood pressure monitor (type RBP-1, supplied by the animal center of Guangdong province academy of TCM) by the tail-cuff method every 2 weeks. The SBP value used for analysis was the average of three records.

Biochemical assessment

After weeks 12 and 20, blood samples were collected from the orbital vein following overnight fasting, and then centrifuged at 3000 rpm/min for 10 min. The supernatant was stored at 4°C and serum levels of total cholesterol (TC), triglyceride (TG), low density lipoprotein (LDL-C), high density lipoprotein (HDL-C), and fasting blood glucose (FBG) were measured using a Cobas8000 automatic biochemical analyzer. Fasting insulin levels were determined by Elisa (Cosabio Ltd. Co.). Insulin resistance (IR) was evaluated in accordance with the homeostasis model assessment index (HOMA-IR) using the following formula: FPG levels (mmol/L) × fasting insulin levels (FIN, mIU/L)/22.5.

Measurement of liver LXRα and β mRNA

At the end of the 20th week, rats were fasted for 12 h, then sacrificed by intraperitoneal injection of chloral hydrate after blood sampling. Liver tissues were collected and frozen in liquid nitrogen. Total RNA was extracted from frozen liver tissues using Trizol reagent, and RNA purity was quantified by agarose gel electrophoresis. LXRα and β and β-actin mRNA levels were determined by real-time fluorescent quantitative PCR using the following primers: LXRα forward 5’-GGAACTAGCTATGCAGTATGTG-3’ and reverse 5’-CCACCGCTATGGCAAATGTG-3’; LXRβ forward 5’-ACAGCCAGCGCTACAACCA-3’ and reverse 5’-GGCAATGAGCAAGGCATACTC-3’, β-actin-specific forward 5’-GGGAAATCGTGCGTGACATT-3’ and reverse 5’-GCGGCAGTGGCCATCTC-3’, producing 133 bp, 165 bp and 76 bp amplified products, respectively. The reaction conditions for PCR were 95°C for 10 min, 60°C for 30 s, 70°C for 30 s for 40 cycles. Gene expression was quantified relative to β-actin and as a fold change to MetS using the -ΔΔCT method.

Statistical analysis

All statistical analyses were performed using SPSS 19.0 software (SPSS Inc, Chicago, IL, USA). Continuous variables were expressed as mean ± standard deviation (SD). Comparisons among groups were performed using analysis of variance with Tukey post-hoc analysis. P-values<0.05 was considered statistically significant.

Results

Establishment of the MetS model

The behavior of control and MetS group model rats did not differ significantly before modeling. However, after 12 weeks, rats in the
MetS model group exhibited gray, dull, dry fur with brown scales, reduced activity and alertness and reduced response to external aural stimuli, produced dry droppings that were thin and/or segmented (Figure 2A). In contrast, rats in the control group did not exhibit these features (Figure 2B).

Before modeling, body weight, abdominal circumference, body length, and SBP value also did not differ between the control and the MetS model rats (all p>0.05). However, at week 12 the high fat diet intake in the MetS model group rats led to significantly lower body weight and abdominal circumference than in control group rats (all p<0.05) (Table 1).

Comparison of physiological parameters before and after CSP treatment

Data are expressed as mean ± SD; *P<0.05 versus control group at the same time point.

Table 2. Comparison of open-field test scores during modeling phase

Data are expressed as mean ± SD; *P<0.05 versus control group at the same time point.

Table 1. Comparison of physiological parameters during modeling phase

Comparison of lipid parameters before and after CSP treatment

Serum levels of TC, TG, HDL-C, and LDL-C did not differ significantly between FI and FC or NI and NC groups before CSP treatment (all p>0.05; Table 3), however CSP decreased serum levels of TC and LDL-C more significantly in FI group than FC group rats (all p<0.05; Table 4).

Comparison of FPG, FIN, and HOMA-IR value before and after CSP treatment

Before CSP treatment, FPG, FIN, and HOMA-IR value did not differ significantly between FI
CSP treatment for MetS

### Table 3. Comparison of physical measurements before and after CSP intervention

<table>
<thead>
<tr>
<th>Time point (week)</th>
<th>Body weight (g)</th>
<th>Abdominal circumference (mm)</th>
<th>Body length (mm)</th>
<th>Systolic blood pressure (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC group</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>311.4 ± 4.7</td>
<td>139.4 ± 5.25</td>
<td>194.1 ± 6.76</td>
<td>181.2 ± 4.94</td>
</tr>
<tr>
<td>20</td>
<td>317.2 ± 5.8</td>
<td>139.5 ± 4.9</td>
<td>194.8 ± 8.3</td>
<td>179.4 ± 8.72</td>
</tr>
<tr>
<td>NI group</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>309 ± 4.32</td>
<td>137.7 ± 4.3</td>
<td>193.5 ± 5.74</td>
<td>178.3 ± 4.81</td>
</tr>
<tr>
<td>20</td>
<td>314.3 ± 5.3*</td>
<td>138 ± 6.3</td>
<td>195.3 ± 5.9</td>
<td>179.4 ± 7.82</td>
</tr>
<tr>
<td>FC group</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>321.1 ± 5.17</td>
<td>146.2 ± 4.34</td>
<td>190.2 ± 5.35</td>
<td>178.3 ± 8.1</td>
</tr>
<tr>
<td>20</td>
<td>337.7 ± 5.38</td>
<td>150.9 ± 4.43</td>
<td>192.5 ± 6.8</td>
<td>178.6 ± 5.34</td>
</tr>
<tr>
<td>FI group</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>317.6 ± 7.37</td>
<td>147.7 ± 4.79</td>
<td>194.8 ± 7.53</td>
<td>178.4 ± 9.06</td>
</tr>
<tr>
<td>20</td>
<td>326.1 ± 6.37*</td>
<td>146.7 ± 4.3*</td>
<td>194.4 ± 8.41</td>
<td>180.5 ± 5.93</td>
</tr>
</tbody>
</table>

Fl, fat diet and CSP intervention; FC, fat diet control group; NI, normal diet and CSP intervention; NC, normal diet control. Data are expressed as mean ± SD; *P<0.05 versus FC or NC group at the same time point.

### Table 4. Comparison of lipids level before and after CSP intervention

<table>
<thead>
<tr>
<th>Time point (week)</th>
<th>TC (mmol/L)</th>
<th>TG (mmol/L)</th>
<th>HDL-C (mmol/L)</th>
<th>LDL-C (mmol/L)</th>
</tr>
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<tr>
<td>NC group</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>1.36 ± 0.18</td>
<td>0.37 ± 0.1</td>
<td>0.48 ± 0.04</td>
<td>0.16 ± 0.01</td>
</tr>
<tr>
<td>20</td>
<td>1.39 ± 0.18</td>
<td>1.01 ± 0.1</td>
<td>0.82 ± 0.04</td>
<td>0.3 ± 0.23</td>
</tr>
<tr>
<td>NI group</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>1.29 ± 0.55</td>
<td>0.55 ± 0.23</td>
<td>0.51 ± 0.06</td>
<td>0.14 ± 0.03</td>
</tr>
<tr>
<td>20</td>
<td>1.63 ± 0.36</td>
<td>0.65 ± 0.34</td>
<td>0.67 ± 0.05</td>
<td>0.19 ± 0.1</td>
</tr>
<tr>
<td>FC group</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>2.71 ± 0.18</td>
<td>1.01 ± 0.46</td>
<td>0.49 ± 0.06</td>
<td>1.12 ± 0.18</td>
</tr>
<tr>
<td>20</td>
<td>2.57 ± 0.19</td>
<td>1.08 ± 0.07</td>
<td>0.82 ± 0.03</td>
<td>1.27 ± 0.19</td>
</tr>
<tr>
<td>FI group</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>2.78 ± 0.21</td>
<td>1.09 ± 0.16</td>
<td>0.84 ± 0.05</td>
<td>1.33 ± 0.24</td>
</tr>
<tr>
<td>20</td>
<td>2.34 ± 0.20*</td>
<td>0.88 ± 0.25</td>
<td>0.53 ± 0.04</td>
<td>0.97 ± 0.17*</td>
</tr>
</tbody>
</table>

TC, total cholesterol; TG, triglyceride; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; Fl, fat diet and CSP intervention; FC, fat diet control group; NI, normal diet and CSP intervention; NC, normal diet control. Values are mean ± SD; *P<0.05 versus FC or NC group at the same time point.

### Table 5. Comparison of FPG, FIN, and HOMA-IR value before and after CSP intervention

<table>
<thead>
<tr>
<th>Time point (week)</th>
<th>FBG (mmol/L)</th>
<th>FIN (pmol/L)</th>
<th>IR-HOMA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fl group</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>14.28 ± 3.29</td>
<td>26.33 ± 4.42</td>
<td>16.39 ± 4.09</td>
</tr>
<tr>
<td>20</td>
<td>12.90 ± 1.21</td>
<td>22.20 ± 5.47</td>
<td>12.95 ± 4.71*</td>
</tr>
<tr>
<td>FC group</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>14.65 ± 0.31</td>
<td>25.87 ± 4.20</td>
<td>16.88 ± 2.95</td>
</tr>
<tr>
<td>20</td>
<td>15.23 ± 3.76</td>
<td>27.92 ± 10.74</td>
<td>17.77 ± 4.00</td>
</tr>
<tr>
<td>NI group</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>5.75 ± 0.35</td>
<td>21.63 ± 6.21</td>
<td>5.56 ± 1.68</td>
</tr>
<tr>
<td>20</td>
<td>7.91 ± 1.97</td>
<td>22.51 ± 4.53</td>
<td>7.92 ± 2.41</td>
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<tr>
<td>NC group</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>5.96 ± 0.89</td>
<td>22.75 ± 3.28</td>
<td>5.97 ± 0.9</td>
</tr>
<tr>
<td>20</td>
<td>7.47 ± 2.88</td>
<td>25.92 ± 6.08</td>
<td>8.37 ± 3.34</td>
</tr>
</tbody>
</table>

FBG, fasting blood glucose; FIN, fasting insulin; HOMA-IR, homeostasis model assessment index. Values are mean ± SD; *P<0.05 versus FC or NC group at the same time point. Fl, fat diet and CSP intervention; FC, fat diet control group; NI, normal diet and CSP intervention; NC, normal diet control.

In the current study, we first developed a MetS Liver Qi stagnation model in male SHR via high-fat, high-sucrose diet in combination with chronic restrained stress for 12 weeks. Following successful establishment of this MetS model, we assessed the effect of 24.8 g/kg CSP per day on the development of MetS. Our results indicate that CSP attenuated high-fat, high-sugar diet-induced obesity by reducing body weight and abdominal circumference. We also observed that CSP significantly reduced serum levels of TC and LDL-C in MetS model rats, reduced HO-MIR-IR scores in FI group rats, and increased liver LXRα mRNA expression in FI group rats. However, CSP had no obvious effects on the serum FBG level and SBP significantly in FI group than FC group rats (all p<0.05; Table 5).

Comparison of liver LXR α and β mRNA expression before and after CSP treatment

As shown in Figure 3A and 3B, liver LXRα or LXRβ mRNA levels did not differ significantly between Fl and FC (all p>0.05) before CSP treatment. CSP treatment significantly increased liver LXRα expression in FI group rats (p<0.05), but did not significantly affect on liver LXRβ mRNA expression in the FC group (p>0.05).

Discussion

In the current study, we first developed a MetS Liver Qi stagnation model in male SHR via high-fat, high-sucrose diet in combination with chronic restrained stress for 12 weeks. Following successful establishment of this MetS model, we assessed the effect of 24.8 g/kg CSP per day on the development of MetS. Our results indicate that CSP attenuated high-fat, high sugar diet-induced obesity by reducing body weight and abdominal circumference. We also observed that CSP significantly reduced serum levels of TC and LDL-C in MetS model rats, reduced HO-MIR-IR scores in FI group rats, and increased liver LXRα mRNA expression in FI group rats. However, CSP had no obvious effects on the serum FBG level and SBP value.
CSP treatment for MetS

Figure 3. The mRNA expression of LXRα (A) and LXRβ (B) in the liver before and after CSP intervention. Data are expressed as mean ± SD (n = 3). *P<0.05 versus FC or NC group at the same time point. FI, fat diet and CSP intervention; FC, fat diet control group; NI, normal diet and CSP intervention; NC, normal diet control.

Insulin resistance plays an important role in the development of MetS. Therefore, the molecular processes involved in insulin resistance are considered drug targets for treatment of MetS. In this study, CSP reduced HOMA-IR, indicating that this formula may represent a promising candidate for management of MetS. Recent studies have indicated the protective effects of CSP in nonalcoholic fatty liver disease in rats with insulin resistance [17].

To explore the mechanisms by which CSP may attenuate MetS, we investigated LXRα and β mRNA expression in the liver. Currently, the contribution of LXR signaling to the pathogenesis of MetS remains unclear. Activation of LXRs signaling was reported to reduce liver glycogen dysplasia and improve insulin resistance.
CSP treatment for MetS

resistance in peripheral tissues [28]. Modulation of LXR and their downstream signaling pathway has been identified as a promising pharmaceutical target for metabolic diseases, particularly cholesterol metabolism [29]. In this study, more significant decreases in the LXRα mRNA expression were observed in the FI and FC group than the FC and FI groups. Treatment with CSP substantially increased liver LXRα mRNA expression, without affecting liver LXRβ mRNA expression in the FI group. However, no similar effect was observed in the normal diet group rats following CSP treatment. These results reveal that the expression of LXRα mRNA is decreased in MetS rats with Liver Qi stagnation, and CSP may increase the expression of LXRα mRNA under pathological conditions.

Some limitations of this study should be noted. First, although our study demonstrates that CSP can attenuate MetS, the time and dose-dependent effects of CSP were not investigated in this preliminary study. Second, considering our preliminary findings, future studies will be required to explore the effect of CSP on downstream effectors of LXRα, and the role of this signaling pathway in regulation of insulin resistance.

This study demonstrates a protective effect of CSP in MetS with Liver Qi stagnation pattern in SHR. The underlying therapeutic mechanisms of CSP may involve up-regulation of liver LXRα mRNA expression.

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Disclosure of conflict of interest

None.

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