Abstract

Obesity is associated with a complex systemic inflammatory state that has been implicated in the development of common, medically important complications, including diabetes, cardiovascular disease and hepatic steatosis. The primary purpose of this study was to test the efficacy of Yin-Chen-Hao-Tang (YCHT) in obesity mice that develop hepatomegaly, neutrophil infiltration and oxidative stress. Female hamsters were fed regular diet (caloric content of 3.4 kcal/g) or high-fat diet (caloric content of 5.3 kcal/g) for fifteen weeks. Liquid chromatography/electrospray ionization time-of-flight mass spectrometry (LC/ESI-TOFMS) was used to clarify the chemical composition of YCHT. Blood samples were collected for biochemistry, inflammatory cytokines, and adiponectin analysis. Endothelial progenitor cells were calculated by flow cytometry at the end of experimental period. Hepatic samples for histopathology, free fatty acid synthesis and oxidative stress parameters by Western blot and RT-PCR. Two-dimensional electrophoresis, MALDI-TOF and LC-MS/MS were performed to identify changeable proteins. The increases in body weight and visceral fat mass were smaller in the YCHT supplemented mice (p<0.05). Plasma triglyceride concentration was significantly lower in the YCHT-treated mice than in the control mice after HF diet administration (p<0.05). This hypoglycemic effect might be associated with potential cardiovascular protective effects of YCHT that enhanced in adiponectin (153±3 v.s. 353±3, ng/ml) and EPCs expression (0.23±0.01 ¼ % v.s. 0.63±0.05%). TNF-α (1700±100 v.s. 1000±105, pg/ml) and MCP-1 (7×10±30 ±32 v.s. 4800±115, pg/ml) were mildly or moderately inhibited by YCHT at a dose of 250 mg/kg/d after 3 weeks of treatment. Its anti-obesity effect might be associated with inhibition of hepatic free fatty acid concentrations (23±3 v.s. 12±2, mmol/L) and elevated the glutathione levels in hepatic tissues. In addition, YCHT up-regulation in the expression of prosenium proliferators-activated receptor gamma protein and mRNA might be responsible for fatty acid synthase mRNA and CPT-1 mRNA expression. Furthermore, we supposed that YCHT action mechanisms might promote senescence marker protein-30 metabolism that increase resistance to hepatic oxidative stress in obesity mice. This study indicates that YCHT at this dose and time course of administration was effective in reducing oxidative stress associated with fatty liver disease progression in obesity mice.

Study design and Hypothesis

Materials and Methods

- **Mice and Chinese herbal medicine (YIN-CHEN-HAO-TANG) treatment**
  Female hamsters were fed regular diet (caloric content of 3.4 kcal/g) or high-fat diet (caloric content of 5.3 kcal/g) for fifteen weeks.

- **Histopathology**
  - Western blotting
  - RT-PCR
  - Flow cytometry analysis and GSH detection
  - Liquid chromatography/electrospray ionization time-of-flight mass spectrometry (LC/ESI-TOFMS)
  - Two-Dimensional Polyacrylamide Gel Electrophoresis (2-DE)
  - MALDI Mass spectrometry analysis
  - Statistical analysis

Results

Figure 1. Liquid chromatography-time-of-flight mass spectrometry (LC-TOFMS) was used to clarify the chemical composition of YCHT. The square extract of YCHT was analyzed by Agilent 6510 Q-TOF. The separated compounds were recorded in both negative and positive ion polarity modes. Further structural information was performed by MALDI-TOFMS. A total of 145 compounds identified in YCHT could be detected in one of the individual herbs, and 87 compounds were investigated with the propagation process. However, we did discover a few minor peaks in the profile of YCHT that were only present in trace amounts and could not be identified. The other hand, most of the abundant compounds in the individual herbs were detected in YCHT as well.

Figure 2. Effect of YCHT on endothelial progenitor cell (EPC) in obesity mice by fluorescent-activated cell sorter analysis. The levels of circulating EPCs, as defined by CD34/VEGFR2 cells. EPCs are substantially decreased to close mice vs the control group (B). There is significant difference phenotypes for YCHT supplement between the other groups.

Table 1. Biochemical analysis of YCHT on obesity mice

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control (n=5)</th>
<th>YCHT (n=5)</th>
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<tbody>
<tr>
<td>Glucose (mg/dL)</td>
<td>120±10</td>
<td>90±10</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>150±15</td>
<td>90±15</td>
</tr>
<tr>
<td>Cholesterol (mg/dL)</td>
<td>200±20</td>
<td>150±20</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dL)</td>
<td>40±4</td>
<td>60±6</td>
</tr>
<tr>
<td>LDL cholesterol (mg/dL)</td>
<td>160±16</td>
<td>100±10</td>
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Figure 3. Detection of cytokine expression from serum of obesity mice and YCHT treatment groups. Cytokine array patterns were scanned, and the intensities of signals were quantitated by densitometry. Serum TNF-α, MCP-1 and adiponectin levels were measured by ELISA kits were purchased from R&D Systems, Minneapolis, MN. Data are presented as mean ± S.E.M.

Figure 4. Light microscopic analysis of liver sections of obesity mice and mice after YCHT administration. Paraffin-embedded sections were stained with H&E. Control (a, b, c), or portal biliary (d, e, f). Original magnification ×400.

Figure 5. Total glutathione (GSSG+GSH) (A) and GSSG/GSH ratio (B) in liver and serum of obesity mice and mice after YCHT treatment. The protein samples were analyzed by Agilent 6510 Q-TOF. Data were presented as mean ± S.E.M.

Figure 6. Effects of YCHT on PPAR-γ activation, leading to the CCA-2 pathway expression. Western blotting was performed for PPAR-γ, total p-PLA2, phospho-p-PLA2, COX-2, and β-actin. YCHT, increase the activation of nuclear PPAR-γ and phosphorylation of p-PLA2, resulting in upregulation of CCA-2.

Figure 7. Amplification of hepatic TNF-α, FAS and CPT-1 mRNA by RT-PCR from livers of mice. Using mRNA equivalents of 10 pg RNA, samples were amplified for 32 cycles using specific primers. GAPDH was used as an internal control (A). (B) Hepatic free fatty acid quantification by ELISA kit was purchased from BioVision Research, CA, USA.

Figure 8. Partial 2D images of liver proteins from obesity mice and animals receiving YCHT treatment. The protein samples were analyzed as-dated under the Materials and Methods. Example of spots representing proteins whose level changed due to the YCHT treatment are highlighted are shown in (B), and the measured protein levels for SMP30 are shown in (C) by Western blotting analysis.

Summary

YCHT action mechanisms might promote senescence marker protein-30 metabolism that increase resistance to hepatic oxidative stress in obesity mice. This study indicates that YCHT at this dose and time course of administration was effective in reducing oxidative stress associated with fatty liver disease progression in obesity mice.

Acknowledgments

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