竹节参降脂复方对小鼠非酒精性脂肪性肝病的作用及机制研究

段丽，刘朝奇，吴利春，石晨，张长城，袁丁，周琴

【摘要】目的研究竹节参降脂复方（ZDS）干预高糖高脂饮食诱导的非酒精性脂肪性肝病（NAFLD）小鼠脂代谢的作用及其可能机制。方法制备竹节参、丹参和山楂提取物，配制成ZDS待用。取雄性昆明小鼠40只，随机分成4组（n=10）：正常对照组、模型组、ZDS低剂量组和ZDS高剂量组。除正常对照组给予常规饮食外，其他3组均饲喂高糖高脂饮食，ZDS高、低剂量组分别给予90mg/kg或30mg/kg的ZDS。喂养5周后，观察小鼠脂质沉积情况及组织形态学改变，以确认小鼠NAFLD模型建立成功，并评价ZDS对脂质沉积的改善作用；采用RT-qPCR、RT-PCR检测小鼠肝脏miR-34a、SIRT1以及脂代谢相关基因FASN、ACC1的相对表达水平，Western blotting检测小鼠肝组织SIRT1蛋白的表达。结果形态学结果显示，与正常对照组相比，模型组小鼠肝脏组织脂质沉积严重，肝脏甘油三酯（TG）水平明显增高（P<0.05）；肝组织miR-34a mRNA水平升高、SIRT1基因表达下降，而脂质合成相关基因FASN、ACC1表达升高（P<0.05）。与模型组比较，ZDS可明显改善肝脏脂质沉积，肝TG含量明显下降（P<0.05）；肝组织miR-34a mRNA及FASN、ACC1基因表达降低，SIRT1基因表达增加（P<0.05）。SIRT1蛋白与其mRNA变化趋势一致。结论ZDS通过miR-34a/SIRT1通路参与了小鼠脂代谢调节，能有效改善高糖高脂饮食引起的肝细胞脂肪变性，为中药复方干预NAFLD提供了新的思路。

【关键词】脂肪肝；非酒精性；竹节参降脂复方；沉默信息调节因子1
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Effects of Panax japonicus hypolipidemic compound on non-alcoholic fatty liver disease in mice and its mechanism

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【Abstract】To investigate the effects of Panax japonicus hypolipidemic compound (ZDS) on the lipid metabolism and its possible mechanism in non-alcoholic fatty liver disease (NAFLD) mice induced by high sugar and fat diet. Methods The extracts of Panax japonicus rhizoma, Salviae Miltiorrhiz radix Et rhizoma and Crataegi Fructus were prepared, and ZDS compound was formulated according to their antioxidant activities. Forty SPF male Kunming mice were randomly divided into four groups (10 each): normal control group, model group, high-dose ZDS-treated group, and low-dose ZDS-treated group. In addition to the mice in normal control group were given conventional diet, the mice in other three groups were fed high-sugar high-fat diet. High-dose and low-dose ZDS-treated group were given 90mg/kg or 30mg/kg of ZDS. After the treatment of five weeks, the histomorphology and lipid deposition of the liver were observed to confirm the establishment of mouse NAFLD model and the improvement of ZDS compound on lipid deposition. The relative expression of miR-34a, SIRT1, and lipid metabolism related genes (FASN, ACC1) was detected by RT-qPCR and RT-PCR. SIRT1 protein expression was detected by Western blotting. Results Compared with the normal group, the morphological results showed hepatic lipid accumulation in the model group was more serious, the levels of triglyceride (TG) and miR-34a in the liver tissue increased significantly (P<0.05), the expression levels of SIRT1 decreased, and the gene of lipid metabolism such as FASN, ACC1 significantly increased (P<0.05). However, compared with the model group, ZDS compound improved hepatic lipid accumulation, liver TG content significantly decreased (P<0.05), liver tissue miR-34a, FASN and ACC1 expressions decreased, while
SIRT1 expression increased (P<0.05). The protein expression of SIRT1 was consistent with its mRNA expression. **Conclusion** ZDS compound can effectively improve liver cell steatosis through the miR-34a/SIRT1 pathway involved in lipid metabolism regulation, thus providing a new idea for early intervention of NAFLD through traditional Chinese compound medicine.

**Key words** fatty liver, nonalcoholic; Panax japonicas hypolipidemic compound; silent information regulator 1

Non-alcoholic fatty liver diseases (NAFLD) are a kind of non-alcoholic and other diseases leading to liver cell steatosis and lipotoxicity as major characteristic of liver pathophysiological processes. NAFLD is a disease that can be cured, and treatment is required for its benign nature. The disease is closely related to obesity and type 2 diabetes. Long-term NAFLD disease can further progress to non-alcoholic steatohepatitis (NASH), which may lead to liver fibrosis, cirrhosis, and even liver cancer [1]. In China, NAFLD patients accounted for more than 20% of the population. In addition to the economic burden of medical care, the concern of how to effectively prevent the progression of liver fibrosis and the development of NASH is still an unsolved problem [2].

SIRT1, also known as Sir2, is a member of the Sir2 family of histone deacetylases. SIRT1 is a NAD-dependent deacetylase that regulates various metabolic and cellular processes. miRs (microRNAs) are non-coding RNAs that regulate gene expression at the post-transcriptional level. The expression levels of miRs in the liver are closely related to NAFLD progression [3]. The expression of SIRT1 is significantly increased in NAFLD patients [4-5], but the specific mechanism of SIRT1/miRs is still unknown.

**Materials and Methods**

**1. Materials and methods**

1.1. Chemicals and reagents: ZDS extract was isolated from the roots of Panax japonicas Maxim. (Araliaceae). The extraction and purification procedures were performed as described previously [6].

**1.2.** Experimental animals: SPF grade Japanese mice (40 mice per group) were purchased from the Animal Center of Beijing Medical University, with an average weight of 20 ± 5 g. Mice were housed in standard cages with free access to food and water under controlled temperature (22 ± 2 °C) and humidity (50 ± 5%) conditions. All mice were acclimated for 1 week prior to the start of experiments. The study was approved by the Animal Ethics Committee of Beijing Medical University, and all experiments were conducted according to the guidelines of the National Institutes of Health for the care and use of laboratory animals.

**Conclusion** This study provides new ideas for the early intervention of NAFLD through traditional Chinese medicine. Further research is needed to elucidate the mechanisms underlying the effects of ZDS on SIRT1 expression and liver function.
表1 PCR引物序列

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer sequence</th>
<th>Product size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GAPDH</td>
<td>Sense: 5'-GGTTGTCTCACCTGCGACTTCAA-3'</td>
<td>134</td>
</tr>
<tr>
<td></td>
<td>Antisense: 5'-CCACCCTGTGTTGATGCCC-3'</td>
<td></td>
</tr>
<tr>
<td>ACC1</td>
<td>Sense: 5'-CCCGAACAGTGGAACTCAGTAT-3'</td>
<td>163</td>
</tr>
<tr>
<td></td>
<td>Antisense: 5'-GCAAAGACCATTAGAGGTAGCCC-3'</td>
<td></td>
</tr>
<tr>
<td>FASN</td>
<td>Sense: 5'-GCATTGCTGATGGAGTCGTG-3'</td>
<td>188</td>
</tr>
<tr>
<td></td>
<td>Antisense: 5'-GGTCTTGGAGATGGCAGAAATC-3'</td>
<td></td>
</tr>
<tr>
<td>SIRT1</td>
<td>Sense: 5'-CCAGAGTCCAAGTTTAGAAGAACCC-3'</td>
<td>103</td>
</tr>
<tr>
<td></td>
<td>Antisense: 5'-TCCTCCGCACAGATTCGGG-3'</td>
<td></td>
</tr>
<tr>
<td>U6</td>
<td>Sense: 5'-GCCCGCTGGCAGTGTCTTAGCTG-3'</td>
<td>79</td>
</tr>
<tr>
<td></td>
<td>Antisense: 5'-GTGCAGGGTCCGAGGT-3'</td>
<td></td>
</tr>
</tbody>
</table>

2.2 HE染色和油红O染色结果

如图1、2所示，HE和油红O染色结果显示，正常对照组小鼠肝细胞排列紧密均匀，肝索围绕中央静脉呈放射状排列；模型组小鼠肝脏细胞肿胀、变圆，有较明显的脂滴，呈空泡状，细胞排列不规则；相比于模型组，ZDS低剂量和高剂量组的肝细胞排列较为紧密，油红染色明显减少，脂肪空泡情况有显著改善。

图1 小鼠肝组织HE染色（×200）

Fig. 1 HE staining of liver tissue of mice (×200)
A. Normal control group; B. Model group; C. ZDS-low group; D. ZDS-high group

表2 小鼠脂质沉积情况（x±s，n=10）

<table>
<thead>
<tr>
<th>Group</th>
<th>Body weight (g)</th>
<th>Epididymal fat weight (mg)</th>
<th>Epididymal fat index (%)</th>
<th>Liver TG (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>40.05 ± 3.01</td>
<td>1.20 ± 0.10</td>
<td>2.99 ± 0.31</td>
<td>22.37 ± 2.08</td>
</tr>
<tr>
<td>Model</td>
<td>42.68 ± 4.33</td>
<td>1.78 ± 0.22</td>
<td>4.14 ± 0.45</td>
<td>27.43 ± 3.20</td>
</tr>
<tr>
<td>ZDS-low</td>
<td>42.34 ± 5.29</td>
<td>1.00 ± 0.11</td>
<td>2.36 ± 0.21</td>
<td>16.85 ± 2.00</td>
</tr>
<tr>
<td>ZDS-high</td>
<td>39.34 ± 2.92</td>
<td>1.06 ± 0.10</td>
<td>2.63 ± 0.29</td>
<td>19.69 ± 2.10</td>
</tr>
</tbody>
</table>

(1)P<0.05 compared with normal control group; (2)P<0.05 compared with model group

2.3 肝脏miR-34a、SIRT1、FASN、ACC1 mRNA表达水平

与正常对照组比较，miR-34a在模型组小鼠肝脏中高表达，ZDS可明显降低miR-34a的表达(P<0.05，图3A)；SIRT1 mRNA在模型组呈现明显降低水平的表达，ZDS可上调SIRT1 mRNA水平的表达(P<0.05，图3B)。RT-PCR结果显示，模型组FASN和ACC1 mRNA表达水平均明显高于正常对照组，而ZDS低、高剂量组表达水平明显低于模型组(P<0.05，图3C、3D)。

2.4 小鼠肝脏SIRT1蛋白表达水平

SIRT1在饲喂高脂高糖饲料的模型组中表达水平明显低于正常对照
组；应用两种剂量的ZDS后，SIRT1表达水平明显上升，其中ZDS高剂量组尤为明显（P<0.05，图4）。

图4 小鼠肝组织中SIRT1的蛋白表达（Western blotting，n=10）
Fig.4 The protein expression levels of SIRT1 in liver tissues of mice (Western blotting, n=10)
N. Normal control group; M. Model group; (1)P<0.05 compared with normal control group; (2)P<0.05 compared with model group

3 讨 论

NAFLD在西方国家的患病率为20%~50%，在我国发病率也逐年增高，成为影响人们身体健康最常见的肝脏疾病。NAFLD与动脉粥样硬化、病态肥胖、高脂血症、胰岛素抵抗等有着密切的联系[1]，因此寻找预防和治疗NAFLD的有效策略十分重要[9]。

传统的中草药在慢性疾病防治中具有独特的优势，大量文献报道了中草药干预高血脂、糖尿病等疾病的显著效果[10-12]。竹节参为五加科人参属植物竹节参(Panax japonicas C. A. Mey.)的干燥根茎，多项研究表明其具有调脂的作用[13]。丹参为唇形科鼠尾草植物丹参(Salvia miltiorrhiza Bunge)的干燥根和根茎，能改善肝功能，对早期肝硬化、肝肺肿大、肝炎等有一定疗效，近年来丹参防治脂肪肝的相关报道呈逐年增加趋势[14]。山楂为蔷薇科山楂属植物山里红(Grataegus pinnatifida Bunge var. Major N. E. Br)或山楂(Grataegus pinnatifida Bunge)及野山楂(Grataegus cuneate sieb et zucc)的成熟果实，具有多种生物学活性，能明显降低总胆固醇和TG水平[15]。本实验模拟人们日常高糖高脂的饮食习惯，在以高糖高脂饮食成功诱导小鼠NAFLD模型的基础上，采用ZDS进行两种剂量的药物干预，发现ZDS可有效纠正NAFLD模型小鼠附脂脂肪组织的增加以及肝脏脂肪的严重沉积，减少脂质在肝脏中的积累。

SIRT1(沉默信息调节因子1)主要分布在细胞核中，其功能是除去乙酰基，使DNA链能够紧密结合在一起，达到基因沉默的目的。研究显示，SIRT1可通过多种途径调节肝脏脂质代谢；SIRT1通过调节腺苷酸激酶(AMPK)和脂肪酸调节元件结合蛋白1(SREBP1)的活性，从而调控脂肪酸合酶(FAS)、乙酰辅酶A羧化酶(ACC)等脂肪酸和TG合成相关基因，影响脂代谢[16]；SIRT1可使组蛋白去乙酰化，以介导脂代谢相关基因的沉默；Song等[17]发现SIRT1还可与FOXO家族等多种靶基因或蛋白相互作用，通过SIRT1-FOXO诱导细胞自噬的信号通路，改善ob/ob小鼠肝脏脂肪沉积。

miRNAs是一类天然存在的非编码小RNA分子，长度为21~25个核苷酸，调控着生物体内约1/3的编码蛋白的基因[18]。最近研究表明，无论是在
体外还是体内模型中，miRNA都不仅调控细胞生长和分化，同时也调控能量和肝脏代谢功能，调节脂肪酸和胆固醇的生物合成[19]。生物信息学预测分析发现，SIRT1为肝组织特异性miRNA——miR-34a的直接作用靶基因，因此关于miR-34a通过SIRT1的介导来影响脂质代谢的研究相当广泛，且均显示miR-34a可以直接下调SIRT1的表达[20]。本实验也证实高糖高脂饮食引起的miR-34a高表达可使下游SIRT1表达降低，引起脂肪代谢相关基因FASN、ACCI等高表达，进而影响脂代谢，而ZDS可通过miR-34a/SIRT1途径有效改善肝细胞脂肪性。本研究结果为防治NAFLD提供了新思路。

【参考文献】