The effects of resveratrol to prevent nonalcoholic fatty liver disease in mice

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Abstract: The aim is to study the prevention mechanism of resveratrol in fatty liver disease mice. Sixty C57BL/6J mice were randomly divided into 5 groups: normal control group (NC), model group (Model), low dose group (10 mg/kg, LR), Middle dose group (30 mg/kg, MR) and high dose group (100 mg/kg, HR), with giving high fat food for 2 weeks, at the same time corresponding dose lavage, blank control group and model group was given the same volume of physiological saline. Measuring the active of alanine aminotransferase (ALT), aspartate aminotransferase (AST), superoxide dismutase (SOD), malondialdehyde (MDA), glutathione (GSH) and the concentration of Low-desity lipoprotein cholesterol (LDL-C), High density lipoprotein cholesterol (HDL-C), Triglycerides (TG) and Total Cholesterol (TC). Pathological conditions of difference groups were observed by H&E staining, and the gene and protein expressions were detected of PPAR-a, Ehhadh, ChREBP1, FAS and GPAT in liver. Resveratrol had significantly decrease serum ALT and AST activity and TG, TC, LDL-C levels in mice fed by high-fat diet ($P < 0.05$, respectively), improve SOD activity and HDL-C concentration ($P < 0.05$, respectively); MDA content was significantly decreased and GSH and SOD were significantly increased in liver tissue ($P < 0.05$, respectively). Pathology results show that the Res could significantly reduce the liver pathology damage degree, show the hepatocyte fatty degeneration and inflammation. In molecular biology, we found that the mRNA and protein expression of PPAR-a, ChREBP1 and Ehhadh were significantly up-regulation and FAS and GPAT gene and protein expressions were significantly reduced in resveratrol treated groups compared with Model group ($P < 0.05$, respectively).
respectively). Resveratrol had dose-dependent relationship in treatment effects. Resveratrol had obvious protective effects in nonalcoholic fatty liver mice. The mechanism might be associated with antioxidant and lipid metabolism, and had dose-dependent.

**Keywords:** resveratrol; nonalcoholic fatty liver disease; preventive action; lipid metabolism; antioxidant
Introduction

Resveratrol (Res) is a kind of dietary polyphenols, which is widely found in red wine, grapes, and peanuts and so on some plants. Res have extensive pharmacological effects, including anti-cancer, anti-oxidation, anti-inflammatory, antiangiogenic, protect liver and so on (1-10). Nonalcoholic fatty liver disease (NAFLD) has become one of the common chronic liver diseases, through simple fatty liver, adipose sex hepatitis and liver fibrosis process, will eventually develop into liver cirrhosis and liver cancer (11-15), the prevention of NAFLD has been widely attention caused by those results. In present study, we established model mice induced by high-fat diet, and observed the prevention of hepatic injury induced by high-fat diet, and discussed the oxidation and lipid metabolism. Provide experimental basis for resveratrol prevention of nonalcoholic fatty liver disease.

Materials and methods

Materials

C57BL/6J male mice, SPF level, 6~8 weeks age, 18~22g. Room temperature was 22ºC~25ºC. Relative humidity was 55%~60%. The mice were purchased from Animal research center, Nanjing Medical University. Res were purchased from Shanghai walk world chemical reagent co., LTD. The antibodies of PPAR-α, Ehhadh, ChREBP1, FAS and GPAT were purchased from Abcam (USA).

Animal model building and drug delivery

The mice were freely dieting for 1 week. 60 mice were randomly divided into 5 groups: Normal control group (NC), high fat feeding Model group (Model), Res dosage groups were divided into Low (LR), Medium (MR) and High (HR) groups. The mice of each group were 10 mice. When the experiment began, the mice of Res treated groups were filled the stomach by corresponding dose of Res (10, 30 and 100 mg/kg·d, measuring the bodyweight once in two days and changing the diets by body weight) at 9:00 am every days, and preventing for 2 weeks. Since 3 weeks in addition to the NC group, the rest of the group are feeding high fat forage (2% cholesterol, 10% lard, 0.3% sodium cholic acid and 87.7% standard feed) in mice, at the same time, RSV group of corresponding dose lavage Res, blank control group and high-fat lavage saline model group (0.9% NaCl), gastric volume of 0.2 mL, for six weeks. Experimental animals free water, record the high-fat food consumption situation
Related parameters of serum and liver tissue

The ALT, AST, SOD, LDL-C, HDL-C, TG, MDA, GSH and TC levels were measured by kit instructions.

HE staining

Taking some liver part with 4% paraformaldehyde fixed, paraffin embedding slices, HE staining, and microscope to observe the pathological changes.

RT-PCR testing

Measurement of gene expression of enzymes related to hepatic glucose and lipid metabolism, β-oxidation and oxidative stress was performed by quantitative RT-PCR analysis. Total RNA was extracted from the liver using a High Pure RNA Isolation Kit (Roche, Mannheim, Germany) according to the supplier’s protocol; the quality and quantity were checked by agarose gel electrophoresis and spectrophotometer. Reverse transcription was performed using a ReverTra Ace-α-cDNA Synthesis kit (Toyobo, Osaka, Japan). Quantitative RT-PCR was performed using the Applied Biosystems 7900HT system, utilizing Power SYBR Green PCR Master Mix (Life Technologies, California, USA) according to the supplier protocol manual, with 18S rRNA used for internal control; To clearly extend lipid metabolism PPAR-α, Ehhadh, ChREBP1, FAS and GPAT were selected. The following primers were used in the current study: 18S rRNA (forward) aagatcgtgcctcagcatatca, (reverse) tggatatgtttctttccttga; PPARα (forward) tcagggtcactaeggtcgtca, (reverse) cccgagactgcgggaagaga; Ehhadh (forward) ggctgtcctcaggaattga; (reverse) cccgagaatcctcgcggt; ChREBP1 (forward) ctggggcactaagggacttcgag, (reverse) gaaagctgatcgtgggctcct; FAS (forward) ttcaagacgaaatgatg; (reverse) attagttgagtatcagagc; GPAT (forward) caacacctccgacgatc; (reverse)gtgacctgattatatcagatca.

Western blot testing

Using RIPA cracking liquid to extract tissue protein in the ice, determine the sample protein concentration, BCA kit on sample after boil protein denaturation, 10% polyacrylamide gel electrophoresis, turn with wet sex membrane will be transferred to the PVDF membrane protein. Using TBST contained 5% skimmed milk powder close after 2 h, respectively, with a diluent dilution of PPAR alpha, Ehhadh, ChREBP1, FAS and GPAT antibody 4°C incubation overnight, wash the film three
times, to join two diluent dilution of horseradish peroxidase labeled 2 resistance, room temperature 2 h incubation, washing liquid membrane after three times to chemiluminescence, using Image Quant LAS 4000 mini imaging system.

Statistical analysis

Data were analyzed using SPSS software version 12.0 J (SPSS Inc., Tokyo, Japan). The results are expressed as mean ± SD. Comparisons of the four diet groups were performed by one-way analysis of variance (ANOVA), and post hoc analyses were used with Tukey’s test for multiple comparisons. P-values were less than 0.05.

Results

TG, TC, AST and ALT in mice serum

TG, TC, AST and ALT concentrations of model group were significantly higher compared with those of NC group (P < 0.05, respectively). Compared with model group, TG, TC, AST and ALT concentrations of Res treated groups were significantly reduced and were significantly dose-dependent (P < 0.05, respectively). The data was shown in Figure 1 and Figure 2.

Figure 1. The TG and TC concentrations in difference groups

Figure 2. The AST and ALT concentrations in difference groups
Figure 2. The AST and ALT concentrations in difference groups

**LDL-C, HDL-C and SOD in mice**

Compared with NC group, LDL-C concentration of Model group was significantly up-regulation \((P < 0.01)\), the HDL-C, SOD and GSH were significantly reduced \((P < 0.01)\). Compared with Model group, the LDL-C and MDA were significantly reduced, the HDL-C was significantly up-regulation in Res treated groups compared with Model group \((P < 0.01\), respectively)\). The hepatic SOD activity was significantly higher in Res treated groups than that in Model group and the effects of Res groups had dose-dependent \((P < 0.01\), respectively)\). The data were shown in Figure 3, Figure 4 and Figure 5.

Figure 3. The LDL-C and HDL-C concentration in mice serum

Figure 4. The SOD activity in hepatic tissues
HE staining

Hepatic slices of each group were observed under microscope after HE staining. The liver of NC group was normal, and the liver cells were normal (Figure 6A). In the model group (Figure 6B), the liver tissue necrosis was found in the tissue, and the lipid lesions were seen in the cytoplasm. The cytoplasm was filled with fat vacuoles (like arrows). In the low dose group (Figure 6C), the liver tissue diffuse hepatic steatosis was relatively reduced, the liver cells are still swollen, and the liver cells can be seen in the cytoplasm of round lipid vacuoles. In the middle dose (Figure 6D) and high dose group (Figure 6E) in the liver tissue cells in the degree of lipid lesions significantly reduced, the size of the lesion was reduced, the cell can be observed in a small number of round lipid vacuoles. The data was shown in Figure 6.
Relative mRNA expression in difference groups

The PPAR-α, ChREBP1 and Ehadh gene expressions of RSV treated groups were significantly up-regulation compared with model group ($P < 0.05$, respectively). However, The Fas and GPAT gene expressions of RSV treated groups were significantly reduced ($P < 0.05$, respectively). The data were shown in Figure 7.
The relative protein expressions in difference groups

The PPAR-α, ChREBP1 and Ehhadh protein expressions of RSV treated groups were significantly up-regulation compared with model group. However, The Fas and GPAT protein expressions of RSV treated groups were significantly reduced. The data were shown in Figure 8.

![Figure 8. The relative protein expression of difference groups](image)

Discussion

With the development of economy, Chinese NAFLD have been increasing incidence in younger age incidence, become a serious threat to people's physical and mental health of the common diseases (16). Therefore, it is necessary to cause high attention to the harm and prevention (17). The pathogenesis of NAFLD is very complex, at present; almost all remain in the hypothesis stage. The theory of "two strikes" has become the main theory to explain the mechanism of its occurrence (18). The first blow is mainly refers to the liver cells and lipid deposition in insulin resistance and lipid metabolism disorder caused by the formation of pure fatty liver; second hit is oxidative stress and lipid peroxidation. Depending on those, improving lipid metabolism and oxidative stress had effects to prevent the occurrence of NAFLD.

Modern researches had shown that resveratrol had anti fatigue, anti-aging, hepatoprotective and beautifying (19). In this experiment, We administered mice by gavage of resveratrol, it can inhibit the high fat diet induced liver decreased expression of MDA increased and the activity of SOD, showed that pine pollen can weaken the high-fat diet induced lipid peroxidation of liver and can reduce the
damage to liver cells. The changes of liver GSH content showed that high fat diet can lead to loss of liver GSH, pretreatment with decreased content of resveratrol can inhibit liver tissue GSH. The related research also confirmed that the dose of resveratrol could reduce the content of MDA and TG in liver tissue of rats with alcoholic liver injury model, increased glutathione (GSH) content, reduce the degree of fatty degeneration of liver tissue, which had obvious protective effects of resveratrol on alcoholic liver injury (20). AST and ALT are the markers of liver injury enzyme, under normal conditions, these enzymes mainly exists in the nucleus and mitochondria will release when cell membrane is damaged, the circulation in the body fluids, so the increase of AST and ALT activity in serum in order to reflect the specific indicator of liver function damage (21,22). In order to explore the mechanism of Res, we carried out further molecular biology research.

Ehhadh, ChREBP1, FAS, and GPAT, included in lipid-metabolism, are transcriptionally regulated by PPAR-α (23-26). We found that the gene expression of PPAR-α and Ehhadh was significantly up-regulated, and FAS and GPAT were significantly down-regulated in the three Res groups with dose-dependent. We hypothesized that Res would promote fatty acid oxidation and increase energy expenditure by enhancing the expression of these enzymes related to β-oxidation and would regulate fatty acid synthesis by reducing the enzymes associated with lipogenesis and enhancing lipolysi. In this study, Res improved the effects of lipid metabolism with dose-dependent.

In conclusion, Res had significant protective effects to improve mice NAFLD injury induced by high fat diet, the mechanism may be related with the anti free radical lipid peroxidation and regulating lipid metabolism, the study can be used as resveratrol provides an important theoretical basis for the utilization of hepatoprotective drugs.

References