

Regulation of Zhiyixiao on the Expression of Visfatin in NAFLD Rats

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ABSTRACT

AIM: To observe the effect of ZhiYiXiao on NAFLD rats' liver pathology and Visfatin levels and to explore the mechanism of NAFLD by "ZhiYiXiao."

METHODS: NAFLD rats were prepared with a high-fat diet. Rats were divided into low, medium, and high doses of ZhiYiXiao treatment groups, YiShanFu positive control group, saline negative control group, and a normal control group. The RT-PCR method was used to detect the expression of Visfatin mRNA in liver tissue, and ELISA was used to detect the expression of Visfatin's protein.

RESULTS: The expression of Visfatin mRNA and protein was significantly lower in liver tissue ($P < 0.01$). However, compared with the YiShanFu group, each dose group showed no significant difference ($P > 0.05$).

CONCLUSION: ZhiYiXiao can regulate the expressions of Visfatin in liver tissue and can reduce the expressions of Visfatin mRNA and protein; its efficacy is similar to YiShanFu ($P > 0.05$). The mechanism of action by ZhiYiXiao may be related to the regulation of the expression of Visfatin in liver tissue.

Key words: NAFLD; Rats; Visfatin

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INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is a pathological syndrome that is related to the environment and metabolic factors, as revealed by hepatic steatosis. The incidence of the disease has been increasing^[1]. Epidemiological survey data showed that NAFLD has become the most prevalent pathology in all chronic liver disease syndromes in Europe and elsewhere; the incidence of liver fibrosis is an estimated 25% of all chronic liver disease syndromes and, of these cases, about 1.5% to 1.8% progress to cirrhosis^[2-4]. In China, NAFLD will soon surpass chronic hepatitis B to become the leading cause of chronic liver disease^[5].

The pathogenesis of NAFLD is not yet fully understood. In traditional Chinese medicine (TCM), NAFLD is described as "hypochondriac," "phlegm," and "accumulation" and is usually caused by stomach damage, endogenous phlegm, poisoning caused by stagnation or emotional stagnation, liver failure catharsis leading to the disorder of Qi movement and blood stasis. NAFLD shows symptoms of accumulation of phlegm and static blood, as well as intermingled deficiency and excess involving the spleen, kidney, and liver. In recent years, some scholars have asserted that adipokines might play an important role in the process of hepatic steatosis^[6-8]. Visfatin has been more extensively studied because it has a similar effect with insulin in reducing blood sugar and impacting fat metabolism^[9-12]. In this study, rats were prepared as NAFLD models by consuming a high-fat diet. We used ZhiYiXiao^[13-15] as an

intervention to clarify the correlation between Visfatin and NAFLD and to explore the pathogenesis of NAFLD and the treatment of its mechanism by traditional Chinese medicine.

MATERIALS

Animals

A total of 60 SD rats, male, weighing 180 ± 10 g, were provided by the Chinese Academy of Sciences Shanghai Experimental Animal Center. Animals were housed in the Zhejiang Chinese Medicine University Experimental Animal Center. The room was maintained at 23°C with 50% humidity. Animals were housed in light and shade each 12h, were randomly assigned to cages, and had free access to water and feed, raising one week after entering the pre-test phase.

Drugs

ZhiYiXiao: Breit, Citrus aurantium, lotus leaf, milk thistle, Alisma, cassia and other raw ingredients were purchased from Hangzhou, Zhejiang Medicines, Hui Chun Tang Hall Square.

YiShanFu (Polyene phosphatidylcholine capsules): Beijing million Ante Pharmaceutical Co. 228mg / tablets, batch number: D1141.

NS: Shapuaisi Zhejiang Pharmaceutical Co., Ltd., batch number: 110313-3.

Equipment

OLYMPUS microscope, OLYMPUS Japanese companies; Hoefer300 electroporation device; MyCycler PCR instrument; BioRadiCyclerIq PCR instrument; Optical Caps (8 Caps/Strip) (Applied Biosystems, Cat # 4323032); Optical Adhesive Covers (Applied Biosystems, Cat # 4311971); US BioRad company adjustable micropipette gun, Thermo Labsystems; -80°C low temperature freezer; Sanyo AR2130 electronic precision balance, Ohaus Corp., Pine Brook, NJ, USA; YDS-3S liquid nitrogen biological containers, four Chuanya Xi machine Factory; PCR tube, Huizhou CSL Plastics Co.

Reagents

M-MLV RTase, Invitrogen; Taq enzyme; Oligod (T) 18Primers; RNase inhibitor; dNTP; 100bp marker; PCR primer synthesis.

METHODS

Model and group

The 60 male SD rats were randomly divided into easy-to-eliminate-fat low, medium, and high dose groups, YiShanfu positive control group, model control group, and a normal control group ($n=10$ in all groups). The control group was fed the normal diet while the rest were provided with a high-fat diet (10% lard, 2% cholesterol, 0.5% bile salts, 5% egg yolk powder, 82.5% normal diet). Animals were fed for 12 weeks after modeling and then were sacrificed.

Route of administration and dosage

Following the experimental animal studies, the equivalent dose was computed of t^{16} (50 kg weight person/200g body weight in rats conversion coefficient $r=0.021$) in order to calculate the equivalent dose of ZhiYiXiao.

During the experiment, ZhiYiXiao low, medium, and high dose groups were fed the equivalent of 3, 6, or 12 times the clinical dose of ZhiYiXiao, respectively, The YiShanFu positive control group was fed the adult clinical dose containing 6 times YiShanFu; the model control group and normal control group were fed with the same capacity group 0.9% saline.

Specimen collection and preservation

The rats were reared to 12 weeks after administration. On their last night, they were not given any food except water for 12h. The next morning a 10% chloral hydrate solution (3 mL/kg) was injected into the abdominal cavity to anesthetize, then heart blood serum was separated and placed in -20°C refrigerator stand. The liver tissue was quickly removed and weighed. Sample blocks of tissue were cut from the middle of the right lobe of liver tissue and then stored in liquid nitrogen to prepare for the extraction of total mRNA.

Index detection

ELISA to detect the expression of Visfatin's protein: Operating in accordance with the kit instructions, Visfatin protein concentration in the liver tissue samples was determined by standard curve.

RT-PCR to detect the expression of Visfatin mRNA: Following kit instructions, the Union of Optimas digital color image microscopy system was used for image analysis and analysis of the regional average gray.

Statistical analysis

SPSS 19.0 for Windows software was used for statistical analysis. Experimental data are below expressed as mean \pm standard deviation ($\pm s$). Statistical analysis included one-way ANOVA and two-sample t-test; an alpha level of $P < 0.05$ was defined as statistically significant.

RESULTS AND ANALYSIS

Expression of Visfatin's protein detected in liver tissue

Compared with the normal group, the expression of Visfatin protein in the liver tissue of the model group was significantly higher than the normal group ($P < 0.05$). Compared with the model group, the ZhiYiXiao each dose group complex hepatic tissue Visfatin protein's expression was significantly lower than the model group ($P < 0.05$). Compared with the YiShanFu group, there were no significant differences in liver tissue Visfatin proteins expression levels of ZhiYiXiao each group ($P > 0.05$, Table 1).

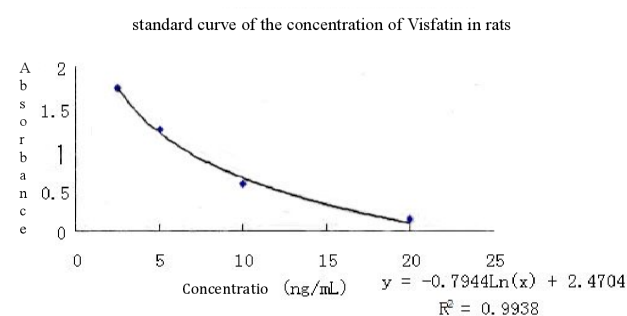
Visfatin mRNA expression levels detected in liver tissue

Compared with the normal group, Visfatin mRNA expression levels in the model group were significantly higher than the normal group

Table 1 Visfatin protein's expression in each group ($\bar{x} \pm s$).

Group	<i>n</i>	Visfatin concentrations (ng/L)
Low Dose	9	$17.31 \pm 0.27^* \Delta \#$
Medium Dose	10	$16.85 \pm 0.44 \Delta \#$
High Dose	9	$16.97 \pm 0.58 \Delta \#$
YiShanFu	10	$17.19 \pm 0.38 \Delta$
Model	10	$18.52 \pm 1.38^*$
Normal	10	16.0215 ± 0.00

vs normal group, * $P < 0.05$; *vs* model group $\Delta P < 0.05$, *vs* YiShanFu group, # $P > 0.05$.

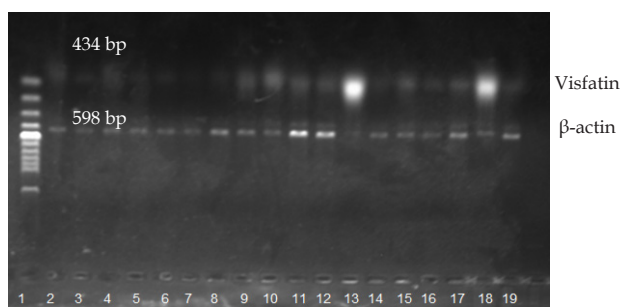


($P < 0.05$). Compared with the model group, the ZhiYiXiao each dose group complex hepatic tissue Visfatin mRNA expression was significantly lower than the model group ($P < 0.01$). Compared with the YiShanFu group, no significant differences in liver tissue Visfatin mRNA expression levels of ZhiYiXiao were observed in each dose group. Taken together, these experimental results showed that the incidence of NAFLD Visfatin participation and development of the model of liver tissue Visfatin mRNA and protein levels were significantly higher than that in normal rats a. And ZhiYiXiao can inhibit within the Visfatin's synthesis and secretion in liver tissues ($P > 0.05$, Table 2).

Table 2 Visfatin mRNA expression in each group ($\bar{x} \pm s$).

Group	n	Visfatin mRNA
Low Dose	9	0.8877 \pm 0.38 Δ #
Medium Dose	10	1.4401 \pm 0.31 Δ #
High Dose	9	1.0923 \pm 0.14 Δ #
YiShanFu	10	1.5377 \pm 0.73 Δ
Model	10	3.0873 \pm 0.56*
Normal	10	0.8663 \pm 0.30

vs normal group, * $P < 0.05$; vs model group $\Delta P < 0.05$, vs YiShanFu group, # $P > 0.05$.



1: Mark;
2, 3, 4: Low-dose group; 5, 6, 7: Medium-dose group;
8, 9, 10: High-dose group; 11, 12, 13: Model group;
14, 15, 16: Normal group; 17, 18, 19: YiShanFu group

CONCLUSIONS

ZhiYiXiao can regulate the expression of Visfatin and can significantly reduce the expressions of Visfatin mRNA and protein in rat liver tissue. ZhiYiXiao has a similar effect to that of YiShanFu. The results of this study suggest that Visfatin involves the development and progression of NAFLD and that ZhiYiXiao may influence Visfatin's synthesis and secretion to improve the biochemical makers of NAFLD. Visfatin has been demonstrated to have a similar effect with insulin to reduce blood sugar, join the fat metabolism and inflammation. Future studies should investigate how Visfatin is involved in the development and p regression of NAFLD through the insulin signaling pathway or other signaling pathways.

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CONFLICT OF INTERESTS

There are no conflicts of interest with regard to the present study.

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