ORIGINAL ARTICLE

Effect of Xuezhikang Capsule (血脂康胶囊) on Serum Tumor Necrosis Factor-α and Interleukin-6 in Patients with Nonalcoholic Fatty Liver Disease and Hyperlipidemia

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ABSTRACT **Objective:** To evaluate the effect of Xuezhikang Capsule (血脂康胶囊) on the serum levels of inflammatory factors such as tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) in patients with nonalcoholic fatty liver disease (NAFLD) and hyperlipidemia, and to explore whether it has anti-inflammatory effect. **Methods:** A total of 84 patients were randomly assigned to two groups with stratified block randomization, the treatment group (42 cases) and the control group (42 cases). They were treated with Xuezhikang Capsule and polyene phosphatidylcholine capsule for twenty-four weeks, respectively. The changes in serum TNF-α and IL-6 were measured by enzyme linked immunosorbent assay before treatment and at the 12th and 24th week. **Results:** Compared with those before treatment, the serum levels of TNF-α and IL-6 significantly decreased in both groups after treatment ($P<0.01$). There was no significant change between the two groups for the treatments at different time points ($P>0.05$) and between the two groups for treatments at the same time points ($P>0.05$). **Conclusion:** Xuezhikang Capsule can inhibit the serum inflammatory factor in patients with NAFLD and hyperlipidemia.

KEY WORDS Xuezhikang Capsule, nonalcoholic fatty liver disease, hyperlipidemia, tumor necrosis factor-α, interleukin-6

In recent years, the concept of inflammation has been highly focused on insulin resistance associated diseases such as obesity, nonalcoholic fatty liver disease (NAFLD), type 2 diabetes mellitus, and hyperlipidemia, etc. Yet a lot of studies have shown that some inflammatory factors such as tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) play important roles in the development of NAFLD, and are highly expressed in NAFLD patients[1-3]. Xuezhikang Capsule (血脂康胶囊) is a new kind of medicine extracted from red-yeast-rice with the effect of regulating blood lipids by lowering serum total cholesterol (TC), triglyceride (TG), and low-density lipoprotein, increasing high-density lipoprotein (HDL-C) and inhibiting fat accumulation in the liver[4-7]. However, whether it has anti-inflammatory effect remains unclear. Therefore, this clinical study was carried out to explore the influence of Xuezhikang Capsule on inflammatory factors in NAFLD patients with hyperlipidemia.

METHODS

Diagnosis and Inclusion Criteria

The diagnosis of NAFLD was referred from the diagnostic criteria revised in February 2006 by the Fatty Liver and Alcoholic Liver Disease Study Group, Liver Disease Association, Chinese Association of Medicine and the Fatty Liver and Alcoholic Liver Group, Society of Heptatology, Chinese Medical Association[8]. The diagnosis of hyperlipidemia was referred from the "Suggestions of Prevention and Management on Blood Lipids Disorders" established in China in 1997[9]. Firstly, subjects with abnormality of blood lipids were instructed to take alimentary control for one month, and only the patients with levels of TC ⩾ 5.72 mmol/L and/or TG ⩾1.70 mmol/L would be included in the study.

Exclusion Criteria

Patients with conditions meeting one of the following criteria were excluded from this study: (1) those with alcoholic liver disease, viral hepatitis,

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autoimmune liver disease, and hepatic adipose infiltration induced by medicines; (2) those with the levels of serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) that were more than 2.5 times the upper limit of the normal level; (3) women in pregnancy or lactation stage; and (4) those with primary cardiovascular, lung, renal and hemopoietic complications as well as psychological diseases.

Clinical Materials

Full clinical records were obtained from 84 patients with confirmed clinical diagnosis of NAFLD with hyperlipidemia, as registered from May 2006 to October 2008 at the First Affiliated Hospital, College of Medicine, Zhejiang University, and without any addiction to alcohol. The patients were randomly assigned to the treatment group and the control group at the ratio of 1:1 according to the sequence of their visiting time in the outpatient department. The randomized digits were produced by the NDST software compiled by SUN Rui-yuan. There was no significant difference in the baseline clinical characteristics between the two groups in terms of age, sex, body mass index, history of NAFLD, history of hyperlipidemia, incidence of hypertension and impaired glucose tolerance (P>0.05, Table 1).

Treatment

The patients in the treatment group were administered with Xuezhikang Capsule (Specification: 0.3 g per capsule and 2.5 mg of lovastatin in every capsule, manufactured by Beijing WBL Peking University Biotech Co., Ltd., China), 0.6 g each time, twice a day orally, while polyene phosphatidylcholine capsule (Specification: 0.228 g per capsule, provided by Beijing Aventis Pharmaceutical Co., Ltd., China) was given to the patients in the control group, 0.456 g each time, three times a day orally. The treatment course was 24 weeks for both groups. Other drugs with the same effect were not allowed to be administered during the administration period.

Items and Methods of Observation

Liver Function, Levels of Blood Lipids, Blood Glucose and Serum TNF-α and IL-6

Peripheral venous blood was collected from the patients before treatment as well as on the 12th and 24th week after treatment. The levels of ALT, AST, γ-glutamyl transpeptidase (GGT), cholinesterase (CHE), TG, TC, HDL-C, and fasting plasma glucose (FPG) were measured with the automatic biochemical analyzer by glucose oxidase method; TNF-α and IL-6 were measured by enzyme linked immunosorbent assay (ELISA) with the kit provided by Shenzhen Jingmei Biotech Co., Ltd., China. The level of serum creatine kinase of the treatment group was measured at the same time.

Adverse Reactions

Patients were observed for adverse reactions during the treatment period.

Statistical Analysis

The statistical analysis results are presented as mean ± standard deviation. The data values were analyzed statistically by ANOVA analysis for repeated measurement data with the SPSS10.0 software. P<0.05 was considered significant.

RESULTS

In the treatment group, two patients dropped out from the study due to intake of other lipid drugs, and one did not want to go on with the study because of diarrhea; another 2 patients were lost during follow-up. In the control group 2 patients were also lost. A total of 37 patients in the treatment and 40 in the control group completed the observation and were analyzed.

Table 1. The Baseline Clinical Characteristics of Patients in the Two Groups

Variable	Treatment (n=42)	Control (n=42)	Statistical value	P value
Age (Yr. , $\bar{x} \pm s$)	54.62±9.67	54.29±10.11	0.154	0.878
Sex (Case, M/F)	20/22	21/21	0.048	0.827
Body mass index (kg/m^2, $\bar{x} \pm s$,)	25.48±2.86	25.19±2.69	0.486	0.628
History of NAFLD (Yr., $\bar{x} \pm s$)	8.12±3.24	8.36±3.21	0.338	0.736
History of hyperlipidemia (Yr., $\bar{x} \pm s$)	10.07±4.82	10.64±4.90	0.539	0.591
Hypertension [Case (%)]	19 (45.24)	16 (38.09)	0.441	0.507
Impaired glucose tolerance [Case (%)]	15 (35.71)	15 (35.71)	0.000	–

Table 2. Comparison of the Liver Function between the Two Groups (U/L, $\bar{x} \pm s$)

Group	Case	Time	ALT	AST	GGT	CHE
Treatment	37	Pre-treatment	61.51 ± 20.80	45.47 ± 13.39	52.59 ± 15.71	9849.16 ± 1237.85
		At the 12th week	44.19 ± 12.72*▲	36.65 ± 9.60*	39.97 ± 11.09*	8787.22 ± 958.83*▲
		At the 24th week	41.76 ± 10.23*△	34.22 ± 8.42*△	37.70 ± 11.38*	8575.76 ± 870.99*△△
Control	40	Pre-treatment	60.50 ± 21.37	45.03 ± 13.57	52.05 ± 15.24	9834.20 ± 1280.86
		At the 12th week	37.70 ± 13.63*	34.60 ± 9.49*	37.08 ± 10.53*	8469.40 ± 973.94*
		At the 24th week	35.28 ± 9.73*△	30.38 ± 9.01*△△	33.75 ± 10.96*△△	8212.13 ± 933.37*△△

Notes: *P<0.01, compared with before treatment in the same group; △P<0.05, △△P<0.01, compared with the same group at the 12th week; ▲P<0.05, compared with the control group at the same time points

Table 3. Comparison of Blood Lipids and FPG between the Two Groups (mmol/L, $\bar{x} \pm s$)

Group	Case	Time	TG	TC	HDL-C	FPG
Treatment	37	Pre-treatment	3.09 ± 1.11	5.89 ± 0.81	1.25 ± 0.27	5.61 ± 0.63
		At the 12th week	1.90 ± 0.58*▲▲	5.15 ± 0.58*	1.47 ± 0.25*▲	5.32 ± 0.52*
		At the 24th week	1.83 ± 0.39*	5.11 ± 0.53*	1.48 ± 0.24*	5.30 ± 0.54*
Control	40	Pre-treatment	3.13 ± 1.18	5.84 ± 0.86	1.27 ± 0.27	5.65 ± 0.74
		At the 12th week	2.35 ± 0.71*	5.26 ± 0.69*	1.42 ± 0.24*	5.34 ± 0.54*
		At the 24th week	2.13 ± 0.48*△△	5.25 ± 0.58*	1.44 ± 0.22*△	5.31 ± 0.49*

Notes: *P<0.01, compared with before treatment in the same group; △P<0.05, △△P<0.01, compared with the same group at the 12th week; ▲P<0.05, ▲▲P<0.01, compared with the control group at the same time points

Comparison of the Liver Function between the Two Groups

Compared with the pre-treatment, the levels of ALT, AST, GGT and CHE were significantly lowered (P<0.01) at the 12th week and at the 24th week after treatment in both groups. The levels of the liver function tests at the 24th week were lower than those at the 12th week (P<0.01, P<0.05) except for GGT (P>0.05) in the treatment group. At the 12th week, the levels of ALT and CHE in the control group were lower than in the treatment group (P<0.05), however, there was no significant difference in the liver function between the two groups at the 24th week after treatment (P>0.05, Table 2).

Comparison of Blood Lipids and FPG between the Two Groups

Compared with the pre-treatment, the levels of TG, TC and FPG were lower and HDL-C was increased in the two groups at the 12th and 24th week after treatment (P<0.01). Compared with the 12th week, there were no significant differences in the blood lipids and FPG at the 24th week (P>0.05) except for TG and HDL-C in the control group (P<0.01, P<0.05). At the 12th week, the level of TG was lowered and the HDL-C was elevated significantly in the treatment group (P<0.05, P<0.01), however, there was no significant difference in the blood lipids and FPG of both groups at the 24th week (P>0.05, Table 3).

Comparison of Serum Levels of TNF-α and IL-6 between the Two Groups

Compared with the pre-treatment, the serum levels of TNF-α and IL-6 were lower in both groups at the 12th and the 24th week (P<0.01). Compared with the 12th week, there was no significant difference at the 24th week (P>0.05). There was also no significant difference at the same time points between the two groups (P>0.05, Table 4).

Table 4. Comparison of Serum Levels of TNF-α and IL-6 between the Two Groups (ng/L, $\bar{x} \pm s$)

Group	Case	Time	TNF-α	IL-6
Treatment	37	Pre-treatment	10.87 ± 3.72	6.79 ± 3.87
		At the 12th week	6.51 ± 1.97*	5.09 ± 2.84*
		At the 24th week	6.36 ± 1.86*	4.94 ± 2.25*
Control	40	Pre-treatment	10.73 ± 3.51	6.81 ± 3.88
		At the 12th week	6.35 ± 2.16*	5.03 ± 2.83*
		At the 24th week	6.23 ± 1.98*	4.89 ± 2.50*

Note: *P<0.01, compared with before treatment in the same group

Adverse Reaction

One patient in the treatment group suffered from diarrhea but was relieved after discontinuing administration of the drug. He later requested to quit

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the study. No obvious adverse reation was found in the other patients during the treatment administration. In the treatment group, the serum levels of creatinine kinase before treatment, at the 12th week and at the 24th week after treatment were 87.70 ± 20.39 U/L, 88.05 ± 21.99 U/L and 88.81 ± 20.20 U/L, respectively, with no significant elevation during the administration (P>0.05).

DISCUSSION

Insulin resistance related diseases such as overweight/obesity, type 2 diabetes and hyperlipidemia, etc. are often accompanied with NAFLD. Insulin resistance is known as one of the important pathogeneses of NAFLD, and is even considered as the pattern of manifestation of insulin resistance in the liver[10]. As insulin resistance related diseases such as obesity, NAFLD and hyperlipidemia are further studied, more and more results show that inflammatory factors such as TNF-α, IL-6, and C-reactive protein participate in insulin resistance related NAFLD[1-3]. Insulin resistance cuts down the potential anti-inflammatory action of insulin and promotes the expression of inflammatory factors and the development of inflammation. They interact with each other.

TNF-α and IL-6 are a type of promotory inflammatory cellular factors with extensive effects. They come from macrophages, lipocytes or endotheliocytes. The physically normal TNF-α and IL-6 levels in the blood of healthy people have the effects of anti-infection and immune function enhancement, but too much of them can injure the tissue. The expressions of TNF-α and IL-6 increase when NAFLD occurs, and they may take part in the occurrence of insulin resistance and play important roles in the development of NAFLD through different ways. The underlying mechanisms may be as follows: (1) TNF-α may inhibit the tyrosine phosphorylation of insulin receptor substrate-1 (IRS-1) to interfere with the receptor signal conduction of insulin and influence the histiocytes' utilization of glucose, and thus insulin resistance[11,12]. (2) TNF-α and IL-6 may inhibit the transport of glucose which is stimulated by insulin through down regulating the expression of lipocytes' glucose transporter-4 (GLUT-4)[11,13]. (3) TNF-α and IL-6 can promote the decomposition of lipocyte and increase fat oxidation, and thus the level of endosomatic free fatty acid increases. Through free fatty acids' inhibitory effect on glucose metabolism (oxadation or non-oxadation), the biological effect of insulin is reduced and insulin resistance occurs. (4) The suppressor of cytokine signaling (SOCS) can inhibit the signal transduction of insulin by inhibiting insulin receptors. Tyrosine phosphorylation of IRS-1 and IL-6 can induce the expression of SOCS-3, which indirectly participate in insulin resistance[14]. TNF-α and IL-6 can cause IR through all kinds of ways and promote the development of NAFLD.

Xuezhikang Capsule, a traditional Chinese medication extracted from Chinese red-yeast-rice, has been regarded as a dietary supplement with lipid-lowering effects and is widely used in the US[15]. Xuezhikang Capsule is rich in hydroxyl-methyl-glutaryl coenzyme A (HMG-CoA) reductase inhibitor lovastatin, all kinds of unsaturated fatty acids, sterol, multi-amino acids and flavonoids. Its effects of regulating blood lipids, decreasing the blood glucose and improving the sensitivity of insulin have been reported in many studies. Polyene phosphatidylcholine capsule concentrated and extracted from soybean was used as the control in this study, and its chemical constitution is similar to endogenous lecithin. They mainly go into the hepatocyte and combine with the cellular and organelle membrane of the liver with its unabridged molecule, playing the effect of repairing and maintaining the morphous and function of the hepatocyte. A lot of studies have shown that polyene phosphatidylcholine has the effect of protecting the hepatocyte, regulating blood lipids and alleviating fatty liver. It is a safe and effective medicine.

In this study, Xuezhikang Capsule has been found not only to protect the hepatic function and regulate the blood lipids of NAFLD patients with hyperlipidemia, but also lower the serum levels of TNF-α and IL-6. The above results show that this product may have potential anti-inflammatory effects through inhibiting the expression of TNF-α and IL-6. Therefore, it attenuates the effect of cytokines and then alleviates the insulin resistance and the degree of fatty liver. However, further studies are needed on how Xuezhikang Capsule works.

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