

Efficacy of Tiopronin in treatment of severe non-alcoholic fatty liver disease

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Abstract. – BACKGROUND: Nonalcoholic fatty liver disease (NAFLD) is a clinicopathologic syndrome of which the main feature is diffuse macrovesicular hepatic steatosis caused by deposition of excessive free fatty acid and triglyceride in liver parenchyma.

AIM: To observe the efficacy of Tiopronin in treatment of severe nonalcoholic fatty liver disease (NAFLD).

PATIENTS AND METHODS: 30 patients with severe NAFLD were treated with Tiopronin for 3 months. 30 healthy people were selected as control. The body mass index (BMI) and plasma levels of endotoxin (ET), leptin, IL-6 and IL-8 were measured before and after treatment.

RESULTS: The serum levels of ET, leptin, IL-6 and IL-8 in severe NAFLD group were significantly higher than those in control group ($p < 0.05$). After treatment with Tiopronin, these indexes were significantly lower than before ($p < 0.05$).

CONCLUSIONS: The intestinal endotoxemia (IETM) occurs in patients with severe NAFLD. Leptin, IL-6 and IL-8 play important roles in pathogenesis of NAFLD. Tiopronin can reduce the levels of ET, leptin, IL-6 and IL-8 for treatment of NAFLD.

Key Words:

Nonalcoholic fatty liver diseases (NAFLD), Tiopronin, Intestinal endotoxemia.

that of alcoholic liver disease (ALD), but the patients have no history of excessive drinking. This disease includes simple NAFLD, nonalcoholic steatohepatitis (NASH), and related hepatic cirrhosis and hepatocellular carcinoma^{1,2}. In China, the incidence of NAFLD is increasing year by year³. One survey in US finds that, the chronic hepatic disease is one of the main causes of global morbidity and mortality⁴. This disease can not only harm liver itself, but also aggravate the risk of diabetes mellitus, coronary heart disease, hypertension and cardiovascular and cerebrovascular disease, seriously endangering people's health.

Recently, the adipocyte-secreted peptide hormone leptin has caused widespread concern, due to its promotion for NAFLD development through a variety of ways. Research finds that, leptin is the independent predictor of NAFLD pathogenesis, and can regulate the energy metabolism through the endocrine, paracrine and autocrine pathway^{5,6}. Therefore, the change of leptin level is closely related to NAFLD, and it may provide a basis for early diagnosis of NAFLD and distinguishing of simple fatty liver disease from steatohepatitis. Harte et al⁶ have observed patients for six months to a year and find that, the lipopolysaccharide (LPS) level increases significantly in patients with NAFLD. LPS acts with liver Kupffer cells and macrophages in adipose tissue to activate NF- β pathway, leading to massive release of inflammatory mediator IL-6 for insulin resistance. In addition, LPS promotes the generation of reactive oxygen and induces oxidative stress, causing body injury⁷. Therefore, looking for a liver function improving agent to effectively reduce intestinal endotoxemia (IETM) and inflammatory mediator, and inhibit hepatic fat accumulation and inflammation via interrupting leptin signaling, may be a new treatment strategy.

Introduction

Nonalcoholic fatty liver disease (NAFLD) is a clinicopathologic syndrome of which the main feature is diffuse macrovesicular hepatic steatosis caused by deposition of excessive free fatty acid and triglyceride in liver parenchyma. It is a metabolic stress-induced liver injury closely related to insulin resistance and genetic susceptibility. The pathological change of NAFLD is similar with

Tiopronin is a glycine derivative containing free thiol through which a variety of pharmacological effects are produced. It reversibly binds free radical to form disulfide, and acts as a free radical scavenger. Tiopronin can inhibit the peroxisome formation in hepatic mitochondria, protect the function of certain specific thiols, increase the amount of small molecular polypeptide on mitochondrial membrane, restore and maintain the content of glutathione, protect liver cell membrane, promote the repair and regeneration of liver cells, participate in protein and carbohydrate metabolism, prevent triglyceride accumulation, improve fat metabolism, thus, improving the liver function⁸. In this paper, 30 patients with severe NAFLD were treated with Tiopronin. The regulatory function of Tiopronin on leptin for improvement in NAFLD was investigated.

Patients and Methods

General Data

Patients in our Hospital from January 2006 to May 2011 were enrolled in this study. This study, conducted in accordance with the declaration of Helsinki, was approved from the Ethics Committee of Shanghai Tenth People's Hospital, Tongji University School of Medicine. Written informed consent was obtained from all participants. According to "Practice Guideline for NAFLD"⁹ established by Liver Diseases Society of Chinese Medical Association in 2006, the diagnostic criteria for NAFLD were as follows: (1) Patients have no history of drinking, or the weekly ethanol intake was below 140 g (male) and 70 g (female). (2) The specific diseases such as viral hepatitis, drug-induced liver disease, total parenteral alimentation, Wilson's disease, and autoimmune liver disease were excluded. (3) The symptoms and signs including fatigue, dyspepsia, liver area secret anguish, and hepatosplenomegaly existed, beside clinical manifestations of primary disease. (4) There were metabolic syndrome related overweight and (or) visceral obesity, fasting hyperglycemia, dyslipidemia, and hypertension. (5) The serum levels of transaminase and γ -glutamyltransferase (GGT) mildly to moderately increase (less than 5 times normal), mainly with the increase of alanine aminotransferase (ALT). (6) The imaging manifestations of liver were consistent with the diagnostic criteria of diffuse fatty liver disease, and the specific requirements for B-ultrasound are as follows: There was echo difference between liver and kidney (liver > kidney), and between near field and

far field (near field > far field); The structure of intrahepatic vein is ambiguous; there was mild or moderate liver enlargement.

Exclusion criteria were as follows: (1) viral hepatitis (hepatitis B and C). (2) autoimmune hepatitis. (3) hepatic cirrhosis. (4) biliary obstruction. (5) long-term drinking, daily alcohol intake over 80 g (male) and 40 g (female) for more than 5 consecutive years. (6) severe infection and diabetes mellitus complications. (7) hereditary diseases including Wilson's disease, α -1 antitrypsin deficiency and hemochromatosis. (8) taking drugs leading to adiposis hepatica, (9) acute inflammation, tumor, diabetes mellitus and hypertension, (10) pathologic change in heart, liver, kidney and gastrointestinal tract.

CT scanning was performed on patients with NAFLD. According to the severity, the disease was divided into mild NAFLD (Liver density decreased; Liver/spleen CT ratio ≤ 1), moderate NAFLD (Liver/spleen CT ratio ≤ 0.7 ; Intrahepatic vessels were not clear), and severe NAFLD (Liver density significantly decreased, and even negative; Liver/spleen CT ratio ≤ 0.5 ; Intrahepatic vessels were clearly visible).

Experimental Methods

30 patients (18 males and 12 females) in severe NAFLD group were treated with 200 mg of Tiopronin (Henan Xinyi Pharmaceutical Co., Ltd., Xinyi City, Guizhou Province, China), 3 times a day for 3 months. During treatment, all patients took low-fat diet, and did proper physical exercise, without using other lipid-lowering or enzyme-inducing drugs. 30 normal people (17 males and 13 females) aged 25-52 years from medical center in our Hospital were selected as control.

Observation items included medical history taking, physical examination (blood pressure, weight, height, waistline, hipline, etc.). The height, weight, waistline (through midpoint of line between the inferior border of costal arch and the highest point of iliac spine) and hipline (greater trochanter of femur) of all patients were measured in the morning under the same conditions (without shoes or hat; fasting). Body mass index (BMI) = weight (kg) / height² (m²). Waistline to hipline ratio (WHR) = waistline (cm) / hipline (cm) (normal: < 0.9 and < 0.85 for male and female, respectively).

All patients fasted overnight, and 8 mL of venous blood was drawn from forearm at 7:00-8:00 on the second day. After centrifugation at 1000 g for 10 min, 4 mL of serum was taken for biochemical test (Roche P800 automatic biochemical analyzer, Japanese). The biochemical indicators for

Table I. Comparisons of age, sex and BMI between two groups.

Group	Age (years)	Sex (male/female)	BMI (kg/m ²)
Control	36.11 ± 8.2	17/13	21.87 ± 1.2
Severe NAFLD	36.2 ± 11.8 ^a	18/12 ^a	31.2 ± 3.6 ^b

Note: ^a $p > 0.05$, compared with control group; ^b $p < 0.05$, compared with control group.

detection included fasting plasma glucose (FBG), hepatitis virus markers, liver function (ALT), triglyceride, and total cholesterol (TC). The remaining serum was store at -20°C in refrigerator. Leptin was detected by radioimmunoassay using DSL-53100 Human Leptin IRMA kit (Headquarters company, St Charles, MO, USA). Endotoxin (ET) was detected by limulus test (chromogenic tripeptide substrate, Shanghai Medical Laboratory, Shanghai, China). Cytokine IL-6 and IL-8 were determined by ELISA method (kits were provided by Jingmei Biotech Co., Ltd. (Shanghai, China).

Statistical Analysis

Statistical analysis was performed using SPSS 13.0 (SPSS Inc., Chicago, IL, USA) statistical software. Data were expressed as mean ± SD. *t*-test was used to analyze the differences between two groups. $p < 0.05$ was considered as statistically significant.

Results

Comparisons of Age, Sex and BMI Between Two Groups

As shown in Table I, there was no significant difference of age and sex between two groups ($p > 0.05$). The BMI in severe NAFLD group was significantly higher than that in control group ($p < 0.05$).

Comparisons of Serum Levels of ET, Leptin, IL-6 and IL-8 Between Two Groups

Table II shows that, the serum levels of ET, leptin, IL-6 and IL-8 in severe NAFLD group were significantly higher than those in control group ($p < 0.05$). After treatment with Tiopronin, these indexes were significantly lower than before ($p < 0.05$).

Discussion

Since NASH has been firstly proposed by Ludwig et al¹⁰ in 1980, with the change of people's dietary pattern and lifestyle, the incidence of NAFLD is increasing year by year. In some patients, NAFLD develops to hepatic fibrosis and hepatic cirrhosis. There is no effective treatment method for this disease until now. At present, the two-strike theory proposed by Dayatal and James¹¹ in 1998 is highly recommended for the pathogenesis of NAFLD. The first strike is insulin resistance, which causes deposition of large amounts of free fatty acid and triglyceride in liver, leading to hepatic steatosis. Studies have suggested by laboratory and clinical data that insulin resistance appears to be an intrinsic defect in NAFLD¹²⁻¹⁴. The second strike is oxidative stress and lipid peroxidation, resulting in hepatocellular injury and inflammation. It can increase the susceptibility of liver cells to apoptosis and necrosis, and promote the occurrence and development of hepatic fibrosis and hepatic cirrhosis¹⁵.

The plasma level of LPS increases in many animal models of NAFLD¹⁶, indicating that IETM occurs during the occurrence and development of NAFLD. ET is an important pathogenic factor, and closely related to NASH. As found in this study, the ET level increases in patients with severe NAFLD. ET can activate the adenosine cyclase on intestinal mucosa, and injure epithelial cell mitochondria and lysosome, leading to cell necrosis at top of intestinal villi and epithelial cell autolysis. In addition, ET can also activate the complement system, blood coagulation system and macrophages to directly or indirectly injure the function of intestinal mucosal barrier. Markel

Table II. Comparisons of serum levels of ET, leptin, IL-6 and IL-8 between two groups.

Group	ET (pg/ug · L ⁻¹)	Leptin (ug/L)	IL-6 (ng/L)	IL-8 (ng/L)
Control	0.0362 ± 0.013	6.6 ± 4.7	4.6 ± 1.7	31.42 ± 6.13
Severe NAFLD (before treatment)	0.0642 ± 0.0286 ^b	21.9 ± 13.32 ^b	20.1 ± 9.2 ^b	73.09 ± 20.38 ^b
Severe NAFLD (after treatment)	0.0387 ± 0.0187 ^a	8.1 ± 5.6 ^a	5.8 ± 2.1 ^a	38.68 ± 7.55 ^a

Note: ^a $p < 0.05$, compared with severe NAFLD group (before treatment); ^b $p < 0.05$, compared with control group.

et al¹⁷ believe that, ET can inhibit fresh enterocyte migration and reduce the effects of cell repair factors. This causes local intestinal mucosa damage and triggers inflammatory cascade, leading to local anemia of intestinal mucosa and intestinal barrier injury. Research of Dumas et al¹⁸ shows that, the increased intestinal permeability in ob/ob obese mice is associated with portal endotoxemia. As found by Miele et al¹⁹, the progress of hepatic injury in patients with NAFLD is related with increased intestinal permeability. In patients with chronic liver disease, the serum levels of inflammatory factors significantly increase, accompanied with the change of intestinal permeability.

This study finds that, with the formation of IETM and elevation of ET level, the leptin level gradually increases, indicating that ET is closely linked to leptin. The course of endotoxemia includes energy metabolism disorders, excessive inflammatory response and suppression of immune function. Therefore, leptin may play a certain role in formation of IETM. As found by Lin et al¹⁵, during the course of IETM, the leptin concentration in plasma increases, leading to enhancement of response of cytokine to ET. Sahai et al²⁰ have studied the induced steatohepatitis and hepatic fibrosis in db/db mice with congenital leptin-receptor deficiency and find that, short leptin receptor isoform and osteopontin play important roles in pathogenesis of NAFLD, indicating that leptin is a risk factor for occurrence of NAFLD. In addition, high concentration of leptin can promote the formation of hepatic fibrosis²¹. Fitzpatrick et al²² have studied 45 patients with NAFLD confirmed by liver tissue puncture and find that, the serum level of leptin in patient increases significantly, and the change of leptin level can be used for distinguishing minimal hepatic fibrosis from hepatic fibrosis. Therefore, leptin is one of initiation factors of hepatic fibrosis, and can increase the serum level of leptin in patients with NASH. It is the independent marker of severity hepatic steatosis. Research shows that, in patients with NAFLD, leptin is positively related to serum level of ALT²³. This is consistent with the study of Labruna et al²⁴. Kashyap et al²⁵ find that, when the serum TG is greater than 150 mg/dl, the possibility of NASH increases by 3.4 times. Leptin is correlated to NAFLD and NASH. The reasons may be that, the amino acid deficiency causes the decrease of apolipoprotein synthesis, affecting triglyceride excretion and metabolism in liver. This indicates that, there is an obstacle in transportation of fat in patients with NAFLD and

NASH. Hyperleptinemia may cause hepatocellular injury, and is closely related to hepatitis and hepatic cirrhosis⁵.

IETM can promote macrophages to secrete cytokines. The levels of cytokine IL-6 and IL-8 are significantly higher in patients with severe NAFLD. They play important roles in the initiation, occurrence and development of steatohepatitis and hepatic cirrhosis in patients with NAFLD. Uygun et al²⁶ find that, leptin resistance is unfavourable for weight control in patients with obesity. It can stimulate macrophages to secrete cytokines such as TNF- α and IL-6, and promote differentiation of stellate cells and fat accumulation. IL-8 can activate neutrophils, causing hepatocyte inflammatory response. In patients with alcoholic liver disease, there is often wide range of neutrophil infiltration in liver and peripheral tissues. The fat accumulation in hepatic cells can stimulate IL-8 production, and participate in inflammatory response, resulting in hepatocyte injury²⁷. As found by Bahcecioglu et al²⁸, the IL-8 level in patients with NAFLD was significantly higher than control, and it is positively related to laboratory examination, liver histological change and severity of liver injury. Results of this study show that, after treatment with Tiopronin, the plasma levels of ET, leptin, IL-6 and IL-8 in patients with severe NAFLD decrease significantly. Tiopronin can reduce ET level, decrease response of leptin to ET, and reduce damage of two-stroke, thus improving liver function. This has provided new thought for clinical treatment of NAFLD.

Conclusions

IETM occurs in patients with severe NAFLD. Leptin, IL-6 and IL-8 play important roles in pathogenesis of NAFLD. Tiopronin can reduce the levels of ET, leptin, IL-6 and IL-8 for treatment of NAFLD.

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Conflict of Interest

The Authors declare that there are no conflicts of interest.

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