Basic Investigations

Effects of Yinchenhao Decoction (茵陈蒿汤) for Non-alcoholic Steatohepatitis in Rats and Study of the Mechanism

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Objective: To observe the effects of Yinchenhao Decoction (茵陈蒿汤) for non-alcoholic steatohepatitis (NASH) in rats and study the mechanism.
Methods: Total 18 male SD rats were randomly divided into a normal control group, a model group and a treatment group, 6 rats in each group. Rats in the model and treatment groups were fed with high-fat forage for 10 weeks to prepare the NASH model, and the rats in the treatment group were administrated with Yinchenhao Decoction from the 6th week for 5 weeks. All rats were sacrificed at the end of the10th week and the samples were collected. Serum alanine aminotransferase (ALT) activity, tumor necrosis factor-α (TNF-α) level, and hepatic triglyceride (TG) and free fatty acid (FFA) contents were determined. Hepatic pathological changes were detected by HE staining.
Results: Serum ALT activity, TNF-α level, hepatic TG and FFA contents, and the fatty deposition in hepatocytes were significantly reduced in the rats of the treatment group.
Conclusion: Yinchenhao Decoction has good therapeutic effects for NASH, protecting the liver function and reducing the fatty deposition in liver, which are possibly related with reduction of FFA content and inhibition of TNF-α expression.

Keywords: *Yinchenhao Decoction; non-alcoholic steatohepatitis (NASH); free fatty acid (FFA); tumor necrosis factor-α (TNF-α)*

In recent years, Chinese herbal compound preparations have become an important way for treatment of fatty liver.[1,2] Classical recipe Yinchenhao Decoction (茵陈蒿汤) has the effects of clearing away heat and promoting diuresis, and activating blood flow to remove blood stasis, which is suitable for the pathogenic characteristic of non-alcoholic steatohepatitis (NASH), retention of damp-heat,[3] with obvious effects of preventing and treating fatty liver,[4] but the mechanism is not clear. Therefore, in the present study, the lipid-reducing and liver-protecting effects of Yinchenhao Decoction in rats of NASH induced by high-fat diet were investigated to expound the mechanism around the two important factors "FFA" and "TNF-α" in pathogenesis.

METERIALS AND METHODS

Experimental Animals

Total 18 SD male rats, sanitary degree, weighing about 120 g, were purchased from Shanghai Xipuebikai Experimental Animal Center Co. Lid, No of certificate of quality: SCXK (Hu) 2007-0005, and raised in the Experimental Animal Center of Medical College of Xiamen University.

Drugs

Yinchenhao Decoction (Oriental Wormwood Decoction) was composed of Yin Chen (Herba Artemisiae Capillaris), Da Huang (Radix et Rhizoma Rhei) and Zhi Zi (Fructus Gardeniae) in the dose ratio of 2:1:1. The crude drugs were purchased from Beijing Tong Ren Tang Health Pharmaceutical Co. Ltd, and The Pharmacy Department of Medical College of Xiamen University gave assistance in drug preparation.

High-Fat Forage

High-fat forage was prepared with 83.25% basic forage, 10% lard, 1.5% cholesterol, 0.2% sodium deoxycholate, 5% sugar, and 0.05% propythiouracil.

Reagents

Triglyceride (TG) kits (Batch No. F001-1) were purchased from Zhejiang Dongou Bioengineering Co. Ltd. ALT (Alanine aminotransferase) (No. C009), Free fatty acid (FFA) (No. 200711033) kits, sodium deoxycholate (No. 20080311) and propythiouracil (No. 20080304) were purchased from Nanjing Jiancheng Bioengineering Institute. Rat Tumor necrosis factor (TNF)-α (No. QRCT-652321EIA\UTL) and ELISA kits were purchased from ADL Company.

Modeling and Administration

At the beginning of modeling, the rats were divided into a normal control group, a model group and a treatment

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group according to the random number table, 6 rats in each group. The rats in the model group and the treatment group were fed with high-fat forage, and the rats in the normal group were fed with normal forage. From the 6th week, the rats in the treatment group were intragastrically administrated with Yinchenhao Decoction in a dose of 1 mL/100 g (0.6 g crude drugs/mL), once a day, for 5 weeks. The rats in the normal control and model groups were intragastrically administrated with equal volume of drinking water in the same period.

Determination of Hepatic TG and FFA Contents, Serum ALT Activity, TNF-α Level and Pathological Examination of the Liver Tissue

Two hundreds mg liver tissue was taken and 3 mL alcohol-acetone solution (1:1) was added, and then homogenized at 3000 r/min, 10 s×3, and poured into a tube with plug and mixed fully, and kept overnight. In the next day, it was centrifuged at 3000 r/min for 15 min at 4 ℃, and 10 μL of the supernatant was used for determination of TG content.

Two hundreds mg liver tissue was taken and 2 mL saline was added, homogenized in ice bath at 10000 r/min, 20 s × 2, and then centrifuged at 3600 r/min, centrifugal radius of 9 cm for 20 min at 4 ℃. The supernatant was used for determination of FFA content with FFA kit.

Serum ALT activity was detected with Lay's method,[5] and TNF-α level with ELISA method.[6]

Pathological examination of the liver tissue was made with HE staining.[7]

Statistical Processing

All data were analyzed with SPSS12.0 software packet. The measurement data were expressed as mean ± standard deviation ($\bar{x} \pm s$). One-way ANOVA and *q*-test were used for comparison.

RESULTS

Hepatic TG and FFA Contents in the Groups (Table 1)

As compared with the normal control group, the hepatic TG and FFA contents in the model group significantly increased ($P<0.01$); and compared with the model group, the TG and FFA contents in the treatment group significantly decreased ($P<0.01$, $P<0.05$)

Serum ALT Activities in the Groups (Table 1)

As compared with the normal control group, the serum ALT activity in the model group significantly increased ($P<0.01$); compared with the model group, the serum ALT activity in the treatment group significantly decreased ($P<0.05$).

Serum TNF-α Content in the Groups (Table 1)

As compared with the normal control group, the serum TNF-α content in the model group significantly increased ($P<0.05$); and compared with the model group, the serum TNF-α content in the treatment group significantly decreased ($P<0.05$).

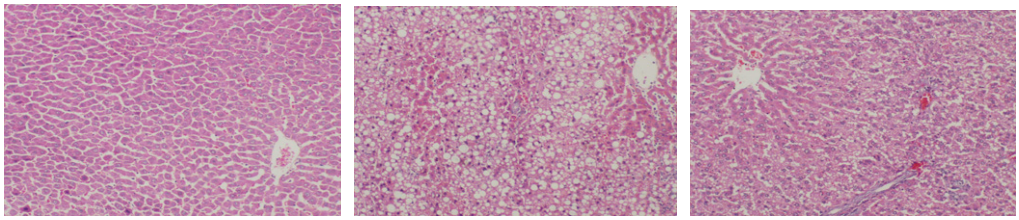
Table 1. Hepatic TG and FFA contents, serum TNF-α levels and ALT activities in the groups of the rats ($\bar{x} \pm s$)

Group	Cases	Hepatic TG (mg/g)	Hepatic FFA (μmol/gprot)	Serum ALT (U/L)	Serum TNF-α (pg/mL)
Normal control group	6	89.2±22.1	154±26	11.3±1.3	3.5±0.2
Model group	6	$727.0\pm41.4^{\Delta\Delta}$	$497\pm43^{\Delta\Delta}$	$22.4\pm3.1^{\Delta\Delta}$	$5.9\pm0.3^{\Delta}$
Treatment group	6	$232.2\pm54.3^{**}$	$333\pm36^{*}$	$14.1\pm2.3^{*}$	$4.1\pm0.3^{*}$

Notes: Compared with the normal group, $^{\Delta}P<0.05$, $^{\Delta\Delta}P<0.01$; Compared with the model group; $^{*}P<0.05$, $^{**}P<0.01$.

Pathological Changes of the Hepatic Tissue in the Groups

According to the guidance of diagnosis and treatment for non-alcoholic fatty liver,[8] in the rats of the normal control group hepatic cells ranged normally, with normal the hepatic cell form, round and large nucleus located at the center, with no accumulation of fat droplet in cytoplasm and no inflammatory cell infiltration in the lobules. In the model group, the liver had obvious fatty degeneration, the hepatic cell swelled and became round, with loose cytoplasm, containing large fat drops, and in a part of the cells the nucleus crowded towards the cell membrane, and inflammatory cell infiltration could be seen. In the treatment group, both the fatty degeneration of liver and inflammatory cell infiltration was obviously alleviated as compared with those in the model group (Figure 1).



A B C

Figure 1. HE staining of the hepatic tissue in the groups (×200). A: Normal control group; B: Model group; C: Treatment group

DISCUSSION

Non-alcoholic lipoidal hepatopathy shows a tendency to rapid increase due to enhancement of people's living standards and changes of living styles and diet structures.[9] A epidemiological survey in Nanning City indicates that the mortality reaches to 28.92%, and easily develops into NASH.[10] The generally recognized and ideal Western medicines for anti-fatty liver is not available at present. However, Chinese medicine shows obvious superiority in preventing and treating fatty liver. TCM holds that the pathogenesis of this disease is failure in governing normal flow of *qi*, dysfunction of the spleen in transport, dampness producing phlegm, retention of damp-heat in the body, stagnation of phlegm in the interior, and blood stasis. As a result, phlegm, heat, blood stasis and dampness jointly give rise to the disorder. Therefore, clearing away heat and promoting diuresis are mainly adopted for its treatment. In the classical recipe of Yinchenhao Decoction, Yinchen (Herba Artemisiae Capillaris) in a large dose, as the monarch drug, has effects of clearing away heat and promoting diuresis; Zhizi (Fructus Gardeniae) as a minister drug has functions of removing pathogenic heat from blood, making damp-heat discharge from urine; and Dahuang (Radix et Rhizoma Rhei) as the assistant drug, functions to purging away the heat and removing blood stasis, inducing the accumulated heat to discharge from stool. Combined use of the 3 drugs may promote smooth and easy urination and defecation, purging away the heat and removing blood stasis, hence fatty liver is cured. Modern pharmacologic researches have demonstrated that Yinchen (Herba Artemisiae Capillaris), Zhizi (Fructus Gardeniae), and Dahuang (Radix et Rhizoma Rhei) may show therapeutic effects for fatty liver respectively by increasing the anti-oxidative action and restoring insulin sensitivity,[11] anti-inflammatory injury and inhibiting fat deposition in liver.[12,13]

In recent years, with the study going deep into the pathogenesis of NASH, it is held that abnormal increases of FFA and TNF-α play a key role in attack of NASH.[14] On the one hand, insulin resistance induces increases of lipodieresis in adipose tissue and FFA synthesis in the liver, and reduction of FFA oxidation and decomposition, leading to disturbance of synthesis and secretion of very low-density lipoprotein, causing accumulation of TG in the liver to form the non-alcoholic lipoidal hepatopathy. And FFA as an amphoteric molecular with high cytotoxicity may cause swelling of cellular mitochondria with increase of its permeability, and degeneration, necrosis and inflammatory cell infiltration of the hepatic cells, finally develop into NASH.[15] On the other hand, the non-alcoholic lipoidal hepatopathy activates Kupffer's cells and adipose cells to secret a large amount of TNF-α, and TNF-α with direct cytotoxicity, leading to injury of the hepatic cells,[16] and inducing inflammation and necrosis of hepatic cells of fatty degeneration, thus develop into NASH.[17] Simultaneously, FFA and TNF-α, as the two important factors in pathogenesis of NASH, have interactive relation. TNF-α can promote lipodieresis of adipose tissue, inducing an increase of FFA; and FFA can induce secretion of TNF-α in hepatic cells, leading to fatty degeneration of the cells and with increase of the toxicity.[18]

The results from this study showed that after the rats were fed with high-fatty forage for 10 weeks, the serum ALT activity and the hepatic TG content significantly increased and the hepatic tissue showed pathological changes of fat degeneration and inflammatory cell infiltration, indicating that high-fat diet can induce NASH pathological changes in the rat. And it was found that serum TNF-α and hepatic FFA contents in the model group significantly increased as compared with those in the normal control group, suggesting that TNF-α and FFA have important effects in attack of NASH. After treatment with Yinchenhao Decoction, serum ALT activity and hepatic TG content significantly decreased, and fat degeneration and inflammatory cell infiltration of hepatic tissue were improved, indicating that Yinchenhao Decoction has obvious lipids-decreasing and liver-protecting effects for NASH. Particularly, serum TNF-α and hepatic FFA contents in the treatment group significantly decreased as compared with those in the model group, suggesting that decrease of FFA and inhibition of TNF-α expression are possibly the important mechanism of Yinchanhao Decoction in prevention and treatment of NASH.

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