Effects of Alum Hepatoprotective on NAFLD Rat's Hepatic Tissue ADP and TNF-α

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ABSTRACT Objective: To investigate the effects of alum hepatoprotective on adiponectin (ADP) and tumor necrosis factor α (TNF-α) in hepatic tissue of the non-alcoholic fatty liver disease (NAFLD) rat. Methods: Forty-eight healthy male Wistar rats were chosen and randomly divided into four groups, with 12 rats in each group. The high fat and high glucose diet was fed to make NAFLD rat models. When the modeling was accomplished after eight weeks, each group would be intervened respectively for four weeks. Then enzyme-linked immunosorbent assay (ELISA) was applied to detect ADP and TNF-α. HE mucus stain was used to observe liver pathologic changes. Results: Compared with that in the normal group, the ADP density in the hepatic tissue of model group decreased obviously (P<0.05) and the TNF-α density increased (P<0.05). Compared with that in the model group, the ADP density in the group which was treated with alum hepatoprotective (alum hepatoprotective group) increased remarkably (P<0.05) and the TNF-α density decreased (P<0.05); Meanwhile, the ADP density in Silybinin group increased obviously (P<0.05) and TNF-α decreased (P<0.05). Compared with that of the Silybinin group, ADP density of the alum hepatoprotective group increased distinctly (P<0.05) and TNF-α had no significant difference (P>0.05). Conclusion: Alum hepatoprotective can significantly increase the ADP density and decrease TNF-α in hepatic tissue, greatly improve hepatic macrovesicular steatosis and reduce inflammatory infiltration, which is probably one action mechanism to treat NAFLD.

Key words: Alum hepatoprotective; NAFLD; Rats; ADP; TNF-α

Chinese Library Classification(CLC): Q95-3, R575.5 Document code: A

Article ID: 1673-6273(2012)15-2855-04

Introduction

Nonalcoholic fatty liver disease (NAFLD) pathogenesis is centered by insulin resistance (IR) and lipidperoxidation (LPO) [1]. The effects of some cell factors such as adiponectin (ADP), tumor necrosis factor α (TNF-α) on this disease pathogenesis have brought increasingly attention. Formulated on the basis of Traditional Chinese Medicine differed fatty liver disease "damp phlegm and blood stasis" theory, alum hepatoprotective showed distinct clinic effects on removing dampness to reduce phlegm, invigorating the circulation of the blood and eliminating stasis [2-5]. This study was to investigate the action mechanism of alum hepatoprotective on treatment of NAFLD through observing its effects on ADP and TNF-α in the NAFLD rat' hepatic tissue.

1 Materials and Methods

1.1 Experimental animals

Forty-eight healthy male Wistar rats, weighing 160± 20 g, were provided by Shandong Lukang Pharmaceutical Group Co. Ltd. Animal qualification No.: SCXK Lu 20090001.

1.2 Experimental Medicines

Alum hepatoprotective (Composition: atractylodes rhizome, officinal magnolia bark, dried tangerine peel, liquorice root, processed pinellia tuber, Indian bread, Hawthorn Fruit, rhizoma alismatis, Turmeric Root Tuber, officinal magnolia bark, semen cassiae torae, alum; provided by Qingdao Infectious Disease Hospital TCM Pharmacy); Silybinin (provided by Tianjin Tasly Pharmaceutical Co., Ltd; State Medical Permitment No.: H20040299); cholesterol and cane sugar (from Guangzhou WHIGA Technology Co., Ltd).

1.3 Experimental reagents and apparatus

Rat adiponectin and TNF-α ELISA kit (American R&D product), Permitment No.: 201108. TB-718E Automatic Biological Tissue Embedding Device (from Taiwei Technology Co., Ltd); RM 2005 Paraffin Slicing Machine (from Shanghai LEICA Equipment Factory); DY89-L Electric Glass Homogenate machine (from Ningbo Xinzhi Keqi Institute); Multiskan Mk3 Microplate Reader (from Thermo).

1.4 Research Methods

1.4.1 Animal grouping and modeling Forty-eight male Wistar rats, after normally fed for a week, were randomly divided into normal control group, model control group, silybinin group and alum hepatoprotective group. Each group had 12 rats. The normal control group was fed with basal diet and other groups high-fat diet[6] with cane sugar (which contained 83 % basal feed, 10 % lard oil, 2 % cholesterol and 5 % cane sugar) for eight weeks. Eight weeks later, two rats from normal control group and three from model control group were chosen and their hepatic tissue obtained

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(Received: 2011-11-25 Accepted: 2011-12-22)

for pathological observation.

1.4.2 Animal dosing The alum hepatoprotective group was orally dosed with 1ml water solution of 2.3 g crude drug per ml density per 100 g body weight twice every day; The Silybinin group was orally dosed with 1ml 22 mg/kg/d dosage Silybinin liquid per 100 g body weight twice every day; the model control group and normal control group were orally dosed with the same volume physiological saline twice every day for four weeks.

1.4.3 Observation targets and inspection methods General condition was detected. Hepatic tissue was detected: a bit of hepatic tissue of the right lobe was sliced and put into 10 % formalin. After paraffin embedding, section and HE mucus stain, the hepatic tissue was observed under light microscope. The pathological diagnosis standard refered to <Guidelines for the diagnosis and treatment of nonalcoholic fatty liver diseases> [7]. Hepatic tissue ADP and TNF-α were detected: hepatic tissue from the same part of liver was taken and made into 10 % tissue homogenate at 4 ℃. The tissue homogenate was centrifuged and the upper clear layer chosen for ELISA.

1.4.4 Statistics analysis SPSS16.0 software was used to analyze the data that was expressed as ($\bar{x}\pm s$) for one-way ANOVA. LSD was used for multiple comparison and Statistically significant leve (P) was 0.05.

2 Results

2.1 Detection of General condition

The normal group had shiny hair, moved swiftly, ate normal diet, had regular urination and bowel movement; while other groups had withered and yellow hair, moved slowly, dilute bowel movement (Fig.1).

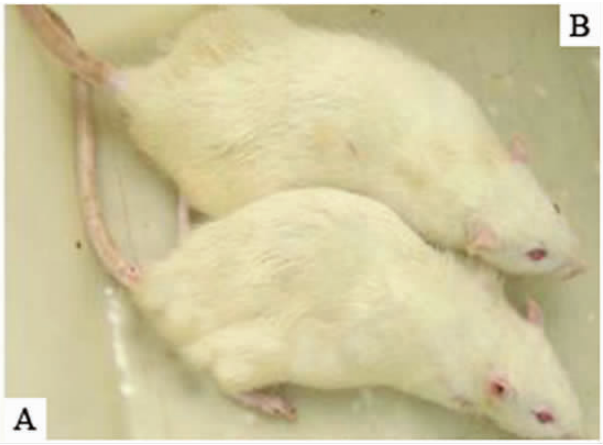

Fig.1 Detection of general condition A: the normal group; B: the model group (12 w)

2.2 Pathologic changes of each group′s hepatic tissue

2.2.1 Visual observation Liver of the normal group was normally sized, rufous colored, soft, and had sharply edged clean section. The model control group's liver markedly was bigger, dull yellow, hard and had blunt edge. Yellow white degeneration could be seen in the section and felt greasy. Liver size, appearance and texture condition of the medicine groups were between that of the model control group and the normal control group.

2.2.2 Pathological observation Hepatic cell cords of the normal control group arranged in radial pattern around hepatic lobule central vein; The the hepatic cells, normally formed, had no fatty degeneration and infiltration. Meanwhile, hepatic cell cords of the model control group arranged in disorder. The hepatic cells in this group swelled and had dark stained nucleus. It had different sized lipid droplet and vacuolar degeneration in the cytoplasm. Inordinate inflammatory cell infiltration occurred at the lobules and portal areas. Compared with that in the model control group, the hepatic cells in the Silybinin group swelled less seriously. There was no lipid droplet in the cytoplasm and the infiltration was slight. Hepatic tissue pathological changes of the alum hepatoprotective group were similar to those of the Silybinin group (Fig.2).

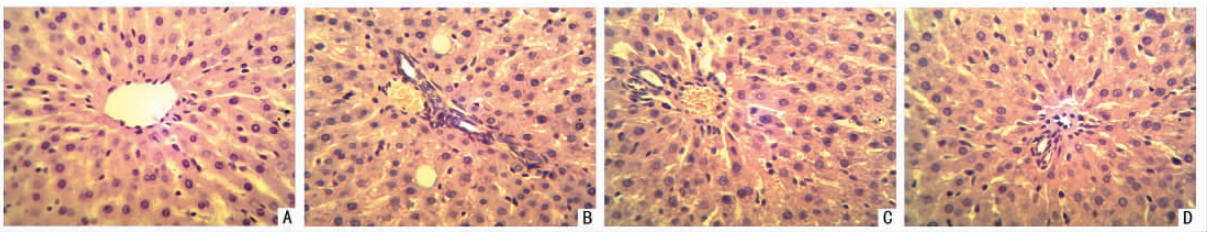

Fig.2 HE stain of rat livers with light microscope (× 400) A: Normal control group; B: Model control group; C: Silybinin group; D: Alum hepatoprotective group

2.3 Hepatic tissue ADP changes of each group

Hepatic tissue ADP density in the model control group was lower than that in the normal control group, P<0.05; Compared with that in the model control group, ADP density in hepatic tissue of the medicine groups all increased but it was lower than that in the normal control group and the differences were significant, P<0.05; ADP density in the alum hepatoprotective group was higher than that in the Silybinin group, P<0.05; The alum hepatoprotective proved to have the best curative effect (Table 1).

2.4 Hepatic tissue TNF−α changes of each group

TNF-α density in the model control group was higher than that in the normal control group, P<0.05; Compared with that in

the model group, TNF-α density in the medicine groups all decreased but it was higher than that in the normal control group and the differences were significant, P<0.05; There was no significant difference for TNF-α density between the alum hepatoprotective group and the Silybinin group, P>0.05 (Table 2).

Table1 The Density of each group's hepatic tissue ADP($\bar{x}\pm s$)

Group	n	ADP(μg/l)
Normal control group	10	33.10± 0.98▲■ ●
Model control group	11	24.45± 0.41★■ ●
Silybin group	11	25.63± 0.81★▲●
Alum hepatoprotective group	11	28.16± 0.93★▲■

Note: compared with the normal control group: ★P<0.05; compared with the model control group: ▲P<0.05; compared with Silybinin group: ■ P<0.05; compared with the alum hepatoprotective group: ● P<0.05.

Table 2 The Density of each group's hepatic tissue TNF-α($\bar{x}\pm s$)

Group	n	TNF-α(ng/l)
Normal control group	10	67.15± 1.20▲■ ●
Model control group	11	231.45± 1.00★■ ●
Silybin group	11	87.94± 1.55★▲
Alum hepatoprotective group	11	88.47± 1.70★▲

Note: compared with the normal control group: ★P<0.05; compared with the model control group: ▲P<0.05; compared with Silybinin group: ■ P<0.05; compared with the alum hepatoprotective group: ● P<0.05.

3 Discussions

The rat model which was made by feeding high fat and carbohydrate diet resembled the human beings in NAFLD causes, biochemistry changes and pathological mechanism [8]. This rat model had high modeling success rate and proved to be the ideal animal model for this disease study.

NAFLD pathogenesis, which belongs to metabolic syndrome [9-11], is centered on IR and LPO. Studies showed that ADP, an endocrine hormone closely related to metabolism, could decrease triglyceride (TG) and free fatty acid (FFA), retrieve IR and prevent inflammatory cell secretion [12]. Jarrar reported that ADP level would be obviously decreased with NAFLD, but pathological dose ADP supply could apparently improve liver cell fatty degeneration and inflammation, which meant that ADP had a close relationship with the occurrence and development of NAFLD [13]. This study showed that ADP density in the model group decreased distinctly (P<0.05); Furthermore, the hepatic-cell fatty degeneration and infiltration in this group were more serious.

After intervened with medicine, ADP density increased and fatty degeneration as well as inflammation was improved accordingly and the alum hepatoprotective turned out to be the most effective in treatment.

TNF-α, a kind of inflammatory cell factor, can cause hepatic cell inflammatory response and injury. It can increase IR, accelerate fat metabolism, produce abundant FFA and expedite fatty degeneration [14]. Results of this study displayed that hepatic tissue TNF-α in the model group increased obviously (P<0.05); After intervened with medicine, TNF-α level decreased (P<0.05) and hepatic tissue fatty degeneration and infiltration improved; There was no significant difference between the two sets of medicine (P>0.05).

Some study stated that ADP had anti-lipid and anti-inflammatory functions, which were opposite to those of TNF-α. These two factors mutually exerted influence on NAFLD through interaction [15].

From the above-mentioned results, it can be concluded that alum hepatoprotective may treat NAFLD by increasing ADP, decreasing TNF-α, and promoting interaction of these two factors to lower IR and improve fatty metabolism as well as anti-inflammation. This study not only confirms the curative effect of alum hepatoprotective on NAFLD, but also clarifies its function approach. Since NAFLD's pathogenesis is complicated, its investigation and action mode of the traditional Chinese medicine are still the further research directions.

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白矾保肝降脂方对非酒精性脂肪肝大鼠肝组织 ADP、TNF-α 的影响

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摘要 目的:观察白矾保肝降脂方对非酒精性脂肪肝大鼠肝组织脂联素(ADP)、肿瘤坏死因子α(TNF-α)的影响。方法:48 只健康雄性 Wistar 大鼠随机分为 4 组,每组 12 只。高脂高糖饲料喂养制备大鼠非酒精性脂肪肝模型。8w 病理显示造模成功后,每组给予相应干预,连续 4w。酶联免疫吸附(ELISA)法测定各组大鼠肝组织中 ADP、TNF-α 浓度,HE 染色观察肝脏病理变化。结果:与正常对照组大鼠比较,模型对照组肝组织中 ADP 浓度明显降低(P<0.05),TNF-α 明显升高(P<0.05);与模型对照组大鼠比较,白矾保肝降脂方组与水林佳组肝组织中 ADP 浓度均明显升高(P<0.05),TNF-α 明显降低(P<0.05);白矾保肝降脂方组大鼠较水林佳组,肝组织中 ADP 浓度显著升高(P<0.05),TNF-α 无显著性差异(P>0.05)。结论:白矾保肝降脂方能显著升高肝组织中 ADP 浓度,降低 TNF-α,明显改善肝细胞脂肪变性,缓解炎性浸润,可能是其治疗非酒精性脂肪肝的作用机制之一。

关键词:白矾保肝降脂方;非酒精性脂肪肝;大鼠;ADP;TNF-α
中图分类号:Q95-03,R575.5 **文献标识码**:A **文章编号**:1673-6273(2012)15-2855-04

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(收稿日期:2011-11-25 接受日期:2011-12-22)