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EXPERIMENTAL STUDY

Protective effect of Yiguanjian decoction against DNA damage on concanavalin A-induced liver injury mice model

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Abstract

OBJECTIVE: To investigate the inhibitory effect of Yiguanjian decoction (YD) on DNA damage in Concanavalin A (Con A)-induced liver injury mice model and to explain the possible mechanism.

METHODS: Totally 120 male BALB/c mice were randomly divided into 6 groups, 20 mice each: normal group, model group, Bifendate group, YD low dose group, YD middle dose group and YD high dose group. Except normal group, liver injury model induced by Con A was established. While modeling, each mouse in YD group was given YD (0.4 mL/20 g per day) by intragastric administration (0.13 g YD for YD low dose group; 0.26 g for YD middle dose group; 0.52 g for YD high dose group). Bifendate group was given Bifendate (0.2 $g \cdot kg^{-1} \cdot d^{-1}$) by gavage. Normal group and model group were fed with same volume of physiological saline daily. After 8 weeks, the serum alanine transaminase (ALT) and aspartate transaminase (AST) were tested. The hematoxylin-eosin staining was used to evaluate the grade of liver inflammation and liver fibrosis stage. Hepatocellular DNA damage was detected by single cell gel electrophoresis technology. The protein expression of tumor necrosis factor-α (TNF-α), Bax and MutT Homolog 1 (MTH1) was detected by western blotting and enzyme linked immunosorbent assay. Bax mRNA and MTH1 mRNA were detected by Real-time Polymerase Chain Reaction (PCR).

RESULTS: YD can improve the degree of liver inflammation and fibrosis in the liver of chronic hepatitis mice, the dose effect relationship is remarkable ($P < 0.05$). YD can reduce liver cell DNA damage. The difference between YD middle dose group and model group was statistically significant ($P < 0.05$). YD middle dose group had decreased the protein expression of TNF-α in the mice liver of immunological liver injury ($P < 0.05$). YD can increase the protein expression of Bax ($P < 0.05$). Compared with normal group, the protein expression of MTH1 was decreased ($P < 0.05$), but there was no statistical significance between YD group and model group ($P > 0.05$). YD can increase the mRNA expression of Bax and MTH1 (both $P < 0.05$).

CONCLUSION: YD can effectively inhibit the DNA damage in immunological liver injury mice, the mechanism may be that it can decrease the TNF-α and increase the Bax and MTH1 expression.

Key words: Drug-induced liver injury; Liver kidney *Yin* deficiency; DNA damage; Concanavalin A; Yiguanjian decoction

INTRODUCTION

China is a high incidence area of hepatitis B virus (HBV) infection, liver cirrhosis and hepatocellular cancer (HCC) are the leading causes of death in the crowd. About 110 000 people died from HCC each year in China, which accounted for 45% of deaths worldwide. And about 90 percent of liver cancer patients were infected with HBV.[1] Therefore, the key measure to reduce the incidence of HCC is active treatment of chronic hepatitis B (CHB), which can prevent CHB developing into cirrhosis or HCC.

The mechanism of CHB leading into HCC has not been fully elucidated, but it is closely associated with the accumulation of DNA damage. HBV can not only cause liver cell DNA damage, but also can directly or indirectly affect the repair system, then block DNA repair and eventually lead to liver cancer.[2-4] How to inhibit DNA damage and promote the repair of it is the main measure, but there is no such drugs in clinical,[5-7] we need further study.

One study found that the active ingredients of Traditional Chinese Medicine (TCM) Tanshinone has some antagonistic action on the DNA damage in peripheral blood lymphocytes of CHB patients induced by H_2O_2.[8] Another study found that grape seed proanthocyanidins (GSP) can antagonize $CdCl_2$-induced hepatic oxidative damage and may inhibit DNA damage or promote DNA repair in rats.[9] Since the pathology of chronic hepatitis B is mainly immunological liver injury, to study TCM on whether it can inhibit or repair the DNA damage in liver cell immune injury is necessary for founding effective medicine to treat HBV patients.

TCM treatment of CHB has achieved remarkable results.[6] Epidemiological findings show that *Yin* deficiency syndrome of liver and kidney is a common but important syndrome of CHB.[10,11] Yiguanjian decoction (YD) is a famous prescription which has the function of nourishing *Yin* and dispersing stagnated liver *Qi*. Clinical practice has proved YD does have good efficacy on treatment of *Yin* deficiency syndrome of liver and kidney of CHB.[12] Experimental studies have found the anti-inflammatory effect of YD is related to its inhibition of hepatic apoptosis.[13] DNA damage is the basis for conversion of hepatitis to HCC but there is no research to indicate whether YD can inhibit and repair DNA damage of liver cells. We choose immunological liver injury in mice as the experimental animal model, to observe the inhibitory effects of YD on DNA damage of Con A-induced immune liver injury, and to explore its molecular mechanism.

MATERIALS AND METHODS

Animals

Totally 120 healthy male BALB/c mice of specific pathogen free (SPF) grade, six-month-old, weighing (20 ± 2) g, were acquired from Vital River Laboratories Animal Technology Co., Ltd. (Certificate of quality No. SCXK [Beijing] 2012-0001, Beijing, China). Mice were housed in the SPF level animal laboratory in Department of Laboratory Animal Science of Capital Medical University, maintained at constant temperature (22-24) ℃ and humidity (30%-45%) under a 12 h light/dark cycle, and all mice had free access to food and water. All experiments were approved by the Experimental Animal Ethics Committee of Capital Medical University.

Drugs

YD consists of Beishashen (*Radix Glehniae*) 9 g, Maidong (*Radix Ophiopogonis Japonici*) 9 g, Danggui (*Radix Angelicae Sinensis*) 9 g, Dihuang (*Radix Rehmanniae*) 20 g, Gouqizi (*Fructus Lycii*) 12 g, Chuanlianzi (*Fructus Toosendan*) 4.5 g. All components were purchased from the Beijing Tongrentang Drugstore(Beijing, China). The aqueous extract of YD was prepared according to the following procedures: These herbs were soaked in 6 times (v/w) distilled water for an hour and then heated to boiling and decocted for 30 min. The filtrate was then collected. The residue was decocted for 20 min with 4 times (v/w) distilled water, and then the filtrate was collected and mixed with the previously collected filtrate. YD was made at a high dose (1.3 g/mL) and a middle dose (0.65 g/mL) and a low dose (0.33 g/mL) and stored at 4 ℃ until use. Bifendate Pills made by Beijing Union Pharmaceutical Factory (Beijing, China), were powdered and dissolved in deionized water at a concentration of 10 mg/mL. Con A made by Sigma-Aldrich Co. LLC. (St. Louis, MO, USA) was dissolved in deionized water at a concentration of 2 mg/mL.

Reagents

RNAprep pure Tissue Kit and Fast Quant RT kit (With gDNase) were purchased from TIANGEN Biotech (Beijing) Co., Ltd. (Beijing, China). SYBR Premix Ex Taq (Tli RNaseH Plus) was purchased from Takara Biotechnology (Dalian) Co., Ltd. (Dalian, China). Mouse tumor necrosis factor-α (TNF-α) enzyme linked immunosorbent assay (ELISA) Kit was purchased from eBioscience, Inc. (San Diego, CA, USA). Anti-Bax and Anti-MutT Homolog 1 (MTH1) antibody were purchased from Abcam Trading Company Ltd. (Cambridge, MA, USA). Anti-TNF-α and anti-β-actin antibodies were purchased from Cell Signaling Technology, Inc. (Danvers, MA, USA). Dylight 680 AffiniPure Goat anti-rabbit IgG and Dylight 800 AffiniPure Goat anti-Mouse IgG were purchased from EarthOx Life Sciences (Millbrae, CA, USA). RIPA Lysis Buffer and protease inhibitors and bicinchoninic acid (BCA) protein concentration assay kit were purchased from Beijing DINOAO Biological Technology Co., Ltd. (Beijing, China).