RESEARCH ARTICLE

Influence of Chongcao Yigan Capsule on Function of Intestinal Flora and Chemoprevention for Patients with Chronic Hepatitis B-Induced Liver Fibrosis

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Abstract

　　Objective: Hapatitis B visus (CHB)-induced fibrosis is a precancerous condition of liver. To explore the influence of Chongcao Preparation (Chongcao Yigan Capsule) on the function of intestinal flora and chemoprevention for patients with CHB-induced liver fibrosis. Methods: A total of 136 patients with CHB-induced liver fibrosis were randomly divided into control group treated with lamivudine (LAM) and research group added with Chongcao Yigan Capsule for totally 48 weeks. The changes of intestinal flora, secretory immunoglobin A (SIgA), serum albumin (ALB), prealbumin (PALB), IgA and IgG at different time points in both groups were observed. Results: Before treatment, there was no significant difference between two groups in each index ($P>0.05$). After treatment, the intestinal flora were evidently optimized in research group than treatment before ($P<0.05$ or $P<0.01$), and were apparently better than those in control group ($P<0.05$ or $P<0.01$); SIgA was obviously increased and ALB, PALB, IgA and IgG were markedly improved in research group than treatment before ($P<0.05$ or $P<0.01$), and were significantly better than those in control group ($P<0.05$ or $P<0.01$). Conclusions: Chongcao Yigan Capsule could regulate the intestinal flora, increase SIgA, serum ALB and PALB concentrations and significantly improve serum IgA and IgG as well as strengthen the immunological function and autologous repair capacity of patients with CHB-induced liver fibrosis.

Keywords: Chronic hepatitis B - liver fibrosis - Chongcao Yigan Capsule - intestinal flora

Introduction

　　Chronic hepatitis B (CHB) is a kind of infectious disease greatly threatening human health, which can develop into liver cirrhosis even hepatic carcinoma (Branchi F, et al., 2014; Shen B, 2014). In 2007, Elsharkawy proposed the developmental axis of hepatitis → liver fibrosis → hepatic carcinoma, which believed that the liver fibrosis was an essential stage for the development from CHB into hepatic carcinoma and was also regarded as the precancerous lesion of hepatic carcinoma (Elsharkawy et al., 2007). Therefore, the treatment and prevention of CHB-induced liver fibrosis has become the primary measure for preventing hepatic carcinoma. Studies on the clinical efficacy of Chinese prescriptions on CHB-induced liver fibrosis have been reported early (Cao, 2010). This study focused on publishing the influence of Chongcao preparation (Chongcao Yigan Capsule) on the intestinal flora of patients with CHB-induced liver fibrosis so as to illustrate the significance of Chongcao Yigan Capsule in the treatment of hepatic diseases from the aspect of nutritional science.

Materials and Methods

Study objects

　　A total of 136 patients with CHB-induced liver fibrosis, who were admitted in our hospital from January 2006 to November 2008 were selected as study objects, were all accorded with the diagnostic criteria of *Prevention and Treatment of Viral Hepatitis* (Colombo et al., 2014). All selected cases had no history of diarrhea or constipation in the near 2 weeks; no administration history of antibiotic and active bacteria agent in the near 1 month; and no history of gastrointestinal surgery. All patients participated in the study voluntarily and were clearly informed of the potential disease aggravation and viral tolerance as well as potential adverse responses and disease progression due to the long-term administration and unauthorized withdrawal of drugs. All Informed Consent Forms were signed and the study was approved by the ethnic committee of Huai'an Chuzhou Hospital and Huai'an First People's Hospital.

Methods

　　Therapeutic methods: The selected objects were

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Table 1. Comparisons of Decreased Value of HBV DNA, IgG, IgA, Serum ALB and PALB at Different Time Points Between Two Groups ($\bar{x}\pm s$)

Therapeutic time	Decreased value of HBV DNA/ $\log_{10}$ Copy·mL^{-1}	IgG/g·L^{-1}	IgA/g·L^{-1}	ALB/g·L^{-1}	PALB/mg·L^{-1}
Research group (n=68)					
Treatment before	—	21.32±4.82	4.34±1.31	29.98±5.42	122.18±49.18
12 w	3.42±1.32**	17.98±2.77##	3.39±1.07**##	34.09±4.07**##	167.94±46.30**##
24 w	3.67±1.41**	16.54±2.11**##	3.04±0.89**##	36.35±3.78**##	192.65±41.05**##
48 w	3.71±1.23**	14.30±1.37**##	1.66±0.49**##	45.24±2.24**##	243.35±41.30**##
Control group (n=68)					
Treatment before	—	21.07±4.48	4.28±1.30	30.01±5.41	125.12±44.30
12 w	2.64±1.10	20.16±4.24	3.92±1.26	31.61±4.00	133.88±43.90
24 w	3.10±1.08#	19.28±3.98#	3.64±1.11##	33.77±3.99##	142.59±41.34#
48 w	3.04±1.10#	17.14±3.07##	3.17±1.04##	35.14±3.70##	152.12±40.88##

Compared with control group, *$P<0.05$, **$P<0.01$; Compared with treatment before, #$P<0.05$, ##$P<0.01$

randomly divided into control group and research group, 68 cases for each group. Control group was treated with single lamivudine (LAM, Approval No.: H20030581, 100 mg/d), on which basis research group was added with Chongcao Yigan Capsule including the extractions of Dongchongciacao (*Cordyceps Sinensis*) 6.7 g, Zhishi (*Fructus Aurantii Immaturus*) 4.5 g, Danshen (*Salvia Miltiorrhiza*) 13.5 g, Tubiechong (*Eupolyphaga Sinensis Walker*) 15 g, Huangqi (*Radix Astragali*) 30 g and Chishao (*Radix Paeoniae Rubra*) 15 g, 0.3 g×5 granules for totally 48 weeks. Other anti-virus, immunoregulatory and various antibacterial agents were forbidden during the treatment, whereas the other therapeutic methods were the same.

HBV marker and SIgA detection: Enzyme linked immunosorbent assays (ELISA) was applied and the reagents were supplied by Beijing Wantai BioPharm Co., Ltd. Quantitative detection of HBV DNA was conducted with American ABI-7000 fluorescent quantitative polymerase chain reaction (PCR) device while the reagents were purchased from Daan Gene Co., Ltd of Sun Yat-Sen University. Hitachi 7180 full automatic biochemical analyzer was used for the detection of biochemical indexes while the reagents were bought from Zhejiang Dongou Biotechnology Co., Ltd.

Bacterial count detection: The initial natural and fresh feces were collected. 1.0 g of fresh feces were measured and put into 10 steriled epoxide (EP) tubes, added with 4.5 mL normal saline (NS) and shaken until they become homogenates. The homogenates were diluted into 10^{-1}, 10^{-2}, 10^{-3}...... and 10^{-10} solutions subsequently. The tips were changed every time when diluted, which were prohibited to touch the tubes and the solutions inside.10 μL of different diluents were collected and inoculated into the selective culture medium of bacteroides, bifidobacterium, lactobacillus, enterobacter and enterococcus, which were then cultured at constant temperature (37°C) for 48 h. According to the bacterial colony count and degree of dilution on the plates, the colony-forming unit (CFU) in the wet quality of each gram of feces was calculated by the formula: CFU/mL= (sample quality+diluted quality)/ sample quality×diluted ratio×bacterial colony count.

Observational indexes

The changes of intestinal flora, secretory immunoglobin A (SIgA), serum albumin (ALB), prealbumin (PALB),

IgA and IgG before and after treatment in both groups were observed.

Safety evaluation

The adverse responses in clinical and laboratory detections during treatment were recorded.

Statistical data analysis

All data was analyzed by PEM3.1 medical statistical software. Measurement data was expressed by mean±standard deviation ($\bar{x}\pm s$) and analyzed by t-test or chi-squared test (x^2) according to the data characteristics of each group with significant level being α=0.05. HBV DNA level was expressed by log kow. The frequency was analyzed with four-form x^2 test. Each bacterial colony count was expressed by 10^x and in normal distribution. The data was in near normal distribution after being transferred by logarithm (lg) with 10 as the base number and expressed by ($\bar{x}\pm s$). The rates of adverse responses were analyzed by x^2.

Results

General data

Of the 68 patients in research group, there were 51 males and 17 females with mean age and HBV DNA baseline value being (52.5±9.1) years and (6.58±1.542)/ $\log_{10}$ copy·mL^{-1}, respectively. Of the 68 patients in research group, there were 50 males and 18 females with mean age and HBV DNA baseline value being (52.8±9.5) years and (6.44±1.41)/$\log_{10}$ copy·mL^{-1}, respectively. There were no significant differences in age, gender and HBV DNA baseline value between two group (x^2=0.079, 0.127, 0.476; $P>0.05$).

Comparisons of decreased value of HBV DNA, IgG, IgA, Serum ALB and PALB at different time points between two groups

Analysis of variance for repeated measurement of serum HBV DNA level indicated that serum HBV DNA level decreased evidently in research group, and was apparently better than that in control group ($P<0.01$). Before treatment, there were no significant differences between two groups in all indexes ($P>0.05$). After treatment, the increases of all indexes were significant in

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Table 2. Comparisons of Intestinal Flora and Immunological Index at Different Time Points Between two Group ($\bar{x}\pm s$)

Therapeutic time	Bacteroides	Bifidobacterium	Lactobacillus	Enterobacter	Enterococcus	SIgA
Research group (n=68)						
Treatment before	8.11±0.41	8.24±0.61	7.26±0.56	8.09±0.50	7.51±0.50	19.94±2.64
12 w	8.59±0.69**##	8.46±0.57**#	7.45±0.46#	8.07±0.39*	7.55±0.32	23.56±3.33**##
24 w	8.68±0.61**##	8.49±0.54**#	7.51±0.42**##	8.02±0.43**	7.61±0.34**	24.19±3.40**##
48 w	9.00±0.56**##	8.74±0.48**##	7.95±0.40**##	7.65±0.45**##	7.79±0.37**##	25.58±3.19**##
Control group (n=68)						
Treatment before	8.15±0.45	8.25±0.39	7.25±0.43	8.10±0.35	7.50±0.35	20.03±3.99
12 w	8.12±0.52	8.12±0.41	7.36±0.35	8.22±0.48	7.59±0.45	20.30±4.04
24 w	8.14±0.60	7.74±0.39##	7.28±0.43	8.34±0.43##	7.95±0.31##	20.62±3.96
48 w	8.18±0.59	7.80±0.43##	7.39±0.40	8.57±0.36##	8.59±0.39##	21.28±2.95#

Compared with control group, *$P<0.05$, **$P<0.01$; Compared with treatment before, #$P<0.05$, ##$P<0.01$

research group than in control group ($P<0.05$ or $P<0.01$), and all indexes increased evidently at each time points after treatment than treatment before ($P<0.05$ or $P<0.01$). There were no significant differences in serum ALB, PALB, IgA and IgG in control group between treatment before and 12 w ($P>0.05$), but the differences were significant 24 and 48 w after treatment than treatment before ($P<0.05$ or $P<0.01$), as shown in Table 1.

Comparisons of intestinal flora and immunological index at different time points between two groups

Before treatment, there were no significant differences in bacteroides, bifidobacterium, lactobacillus, enterobacter, enterococcus and SIgA between two groups ($P>0.05$), which were improved markedly after treatment in research group ($P<0.05$ or $P<0.01$) and than those in control group ($P<0.05$ or $P<0.01$). Enterobacter and enterococcus 12 and 24 w after treatment were improved obviously in research group than treatment before ($P>0.05$), but decreased apparently 48 w after treatment ($P<0.01$). And the differences were significant between two groups ($P<0.05$), as shown in Table 2.

Adverse responses

During treatment, only 1 case of nausea was observed 24 weeks after treatment in control group, which was recovered itself 1 week after the treatment was ended. No other anti-virus drug associated adverse responses were recorded in both groups.

Discussion

CHB is a kind of hepatic disease induced by HBV, which is in blood-borne transmission with high morbidity (Poortahmasebi et al., 2014). Though anti-virus therapies can be used in clinic, most patients with CHB still develop into liver fibrosis or even hepatic carcinoma (Lu et al., 2014). In 2005, the Guidelines for Prevention and Treatment of Chronic Hepatitis B clearly demonstrated that the overall objectives of treating CHB was to maximally inhibit HBV DNA in long term, reduce liver fibrosis and hepatitis and decrease or propone the development of hepatic cirrhosis, decompensation or even carcinoma so as to prolong the survival time and improve the QOL of CHB patients (Uribe et al., 2014). Multiple researches indicated that Chinese medicines had certain

efficacy in the treatment of CHB, whose functions of anti-virus, anti-liver fibrosis, increasing immunological function and improving clinical symptoms could prevent the progression of hepatic carcinoma to some extent (Cao, 2010; Che et al, . 2014).

Chongcao Yigan Capsule concomitant with LAM in the treatment of CHB for 48 w showed that patients' physical signs and HBV DNA baseline decreased evidently while serum ALB and PALB increased markedly in research group, and were significantly better than those in control group ($P<0.05$); serum IgA, IgG as well as intestinal floras and SIgA were apparently optimized in in research group, and were significantly better than those in control group ($P<0.05$). 95.6% (65/68) patients in research group had healthy condition with florid faces after treatment with Chongcao Yigan Capsule, which increased the confidences of patients and their families and improved the virtuous circle of treatment, which were consistent with the therapeutic principles of "comprehensive therapies including anti-virus, immunoregulation, hepatic function improvement and anti-liver fibrosis are necessary for CHB" in other literature reports (Christiansen et al., 2014; Pollack et al., 2014).

Intestinal microecology receives more and more attentions because of its relationship with human health, diseases, therapies for diseases and body repair, which has been recorded in many reports (Floch, 2012). Traditional Chinese medicine believes that the primary function of liver is to regulate qi and blood, and liver fibrosis consists of both blood stagnation and deficiency of healthy qi, therefore, the initial therapeutic principle for liver fibrosis is to invigorating healthy qi to remove blood stasis. Chongcao Yigan Capsule is composed of drugs with effect of invigorating healthy qi to remove blood stasis, such as Dongchongxiacao, Zhishi, Tubiechong, Danshen, Chishao and Huangqi, etc.. The Chinese herbs in the formula contain various dietary fibers that are an important intestinal barrier, which can provide nutrition for colonal microorganism. The fibers can form short-chain fatty acid after bacterial glycolysis, which is necessary in maintaining the nutrition and functional integration of colonal cells. The detection results of this study found that after 12-week treatment, bacteroides, bifidobacterium and lactobacillus increased obviously than treatment before in research group ($P<0.01$), but had no significant differences or decreased in control group, whereas enterobacter and

enterococcus were in the contrary condition in two groups, which was predicated to be in potential association with the regulating function of dominant bacterial colony, and the explicit reason needs to be further explored.

Modern medical examinations demonstrated that $CD4^+$ decreased, $CD8^+$ increased, the ratio of decreased and IgG, IgM and IgA increased significantly in patients with CHB, indicating that CHB patients' bodies could not clear HBV effectively by themselves due to the severe dysfunction of cellular and humoral immunity, which put the disease in a chronic developmental condition (Liang et al., 2014). After application of Chongcao Yigan Capsule, IgG and IgA decreased markedly, suggesting that Chongcao Yigan Capsule had remarkable regulating function on cellular and humoral immunity.

The results of this study verified that from the aspect of nutritional science, Chongcao Yigan Capsule could regulate intestinal flora, improve immunological function and autologous repair ability and effectively inhibit the viral replication so as to reduce HBV DNA level of CHB patients. The detailed mechanism and principle need to be further explored and studied. The results reported in this study have certain limitation due to the limited samples, observational time and funds, which need further clinical verification with expanded samples.

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