Effect of Chishao Chengqi Decoction (赤芍承气汤) on Endotoxin and TNF-a in Patients with Severe Hepatic Diseases*

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Objective: To observe the effect of Chishao Chengqi decoction (CCD) in treating severe ABSTRACT hepatopathy and its influence on serum endotoxin(ET) and tumor necrosis factor α (TNF- α), in order to explore the possible mechanism of CCD in protecting liver cells and in preventing liver failure. Methods: Sixty patients suffering from hepatopathy were divided into the treated group and control group randomly, 30 in each group. They were treated with comprehensive treatment, including hepatocyte growth-promoting factors, thymosin, Transmetil and albumin. CCD was given to the treated group additionally. The therapeutic effects were observed and the changes of some biochemical criteria, including alanine transaminase (ALT), aspartate aminotransferase (AST), total bilirubin (TB), albumin (ALB) as well as such parameters as prothrombin activity (PTA), serum levels of ET and TNF- α were all detected respectively before treatment and after treatment. Results: In the treated group, 8 patients was clinically cured after treatment, 11 were markedly alleviated, 7 improved and 4 remained unchanged, while in the control group, the respective numbers were 5, 8, 8 and 9. The total effective rate of the treated group was significantly better than that of the control group by (P < 0.05). ET and TNF- α levels in patients were significantly higher than the normal range before treatment, and they were lowered after treatment. Comparison of the effect between the two groups showed significant difference (P < 0.05), with that in the treated group better than that in the control group. Conclusion: CCD decoction could reduce the production and releasing of ET and TNF- α in severe hepatopathy patients, which might be one of its therapeutic mechanisms.

KEY WORDS severe hepatic disease, Chishao Chengqi decoction, endotoxin, tumor necrosis factor a

Clinical observation and experimental study of Chishao Chengqi decoction (赤芍承 气汤, CCD) in treating severe hepatopathy have been carried out by the authors in recent years, and the results showed that CCD has the effects of protecting liver cell and antagonizing liver failure^(1,2). In order to explore further its mechanism, the changes of serum endotoxin (ET) and tumor necrosis factor α (TNF- α) in hepatopathy patients after CCD treatment were observed.

METHODS

Clinical Materials

All the 60 severe hepatopathy patients observed were in-patients of Shenzhen East-Lake Hospital, with hospitalized course \geq 10 days, manifesting mainly severe jaundice, hepato-encephalopathy, ascites, purpura, etc., and their diagnosis conforming with the diagnostic standard revised on the 5th National Conference of Infectious and Parasitic Diseases, 1995⁽³⁾. They were divided randomly, according to the order of hospitalization time, into two groups. The 30 patients in the treated group were 28 males and 2 females, with mean age of 36.7 ± 8.8 years; their diagnosis was liver cirrhosis (LS) in 12 patients, chronic hepatitis B of the severe degree (CHB) in 9 and severe chronic hepatitis B (SCH) in 9. The 30 patients in the control group were 29 males and 1 female, with mean age of 37.6 ± 10.2 years and the diagnosis of LS in 11 patients, CHB in 10 and SCH in 9. The two groups were similar in sex, age, blood figure and liver function with insignificant statistical difference (P > 0.05), and so were comparabal.

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Treatment

Conventional comprehensive treatments for liver-protection, juandice abating and function supporting were applied to both groups, using hepatocyte growth-promoting factors, thymosin, Transmetil and albumin. CCD, consisting of red peony root 60 g, magnolia bark 15 g, immature bitter orange 15 g, Glauber's salt 6 g, rhubarb 10 g, was given to the treated group additionally, one dose per day, which was decocted with water, and taken orally by twice or more times, half an hour after meals. Dosage of rhubarb and red peony root in the recipe may be modified individually to make the patients' fecal discharge 3-7 times per day with mushy stool. Administration of CCD lasted for 2 weeks, and then, the prescription of Chinese herbal medicine applied may be changed according to Syndrome Differentiation.

Items and Methods of Observation

1. TNF- α activity and blood level of ET were determined before and after the 2 weeks of treatment. For TNF- α , ELISA double antibody sandwich method (with the kit purchased from Jingmei Bio-medical Co.) was adopted, and for ET azo color-developing process (with the limulus kit purchased from Shanghai Institute of Medical Laboratory Examination) was adopted. Specially appointed persons from Department of radio-immunology, Shenzhen Institute of Hepatopathy performed the tests according to instruction.

2. Biochemical criteria, including alanine transaminase (ALT), aspartate aminotransferase(AST), total bilirubin(TB), albumin(ALB), total cholesterol(TC), and prothrombin activity(PTA) were measured before treatment and after 1, 3 and 5 weeks of treatment. PTA was tested using STA Compact blood coagulometer(from France) and the others using Beckman auto-biochemical analyser type CX-5(from America).

Statistical Analysis

Data were expressed by mean \pm standard deviation. *Ridit* test, variance analysis, *t*-test, and χ^2 test and correlation regression analysis were conducted bilaterally.

RESULTS

Comparison of Clinical Effects

Based on the Standard for Therapeutic Effect Evaluation formulated by the Branch of Hepatopathy Diseases, Chinese Association of $TCM^{(4)}$, the clinical therapeutic effect were classified into 4 grades: clinically cured, markedly effective, improved, and ineffective.

In the treated group, the result was clinical cured in 8 patients, markedly effective in 11, improved in 7 and ineffective in 4 (including one case get discharging himself voluntarily). The corresponding data in the control group were 5, 8, 8, 9 (including 3 cases get discharging himself voluntarily) respectively. Comparison between the two groups in total effective rate showed significant difference by *Ridit* test (P < 0.05).

Effects on Biochemical Criteria

As shown in Table 1, the level of ALT, AST and TB began to reduce, and ALB, TC, and PTA began to increase after being treated for 2-3 weeks in the treated group, but in the control group, these improvements took place on 4-5 weeks later, comparison between the two groups showing significance different in most of the criteria (P < 0.05).

Comparison of TNF-a Activity and ET Content

As Shown in Table 2, the TNFactivity and ET content were higher than the normal range in all the patients before treatment, and showed positive correlation between them (r = 0.425, P < 0.01), with the regressive equation Y = 33.64 + 519.64X, and the levels were in SCH > in CHB>in LS, and significantly higher in the

| Group | | n | ALT(U/L) | AST(U/L) | TB(µmol/L) | ALB(g/L) | TC(mmol/L) | PTA(%) |
|---------|----------------|----|-------------------|--------------------------|-------------------|--------------------|-----------------|-----------------|
| Treated | BT | 30 | 538.8±129.6 | 462.7±143.5 | 257.3 ± 123.4 | 30.07±3.29 | 1.67 ± 0.49 | 41.7±15.1 |
| | 1W.AT | 30 | 513.8 ± 138.3 | 368.5 ± 120.9 | 366.8 ± 102.7 | 27.35 ± 3.17 | 1.39 ± 0.37 | 36.3 ± 13.6 |
| | 3W.AT | 28 | 230.9±75.2*△ | 187.6±97.4*△ | 311.7±131.7* | 30.87±5.40* | 1.79 ± 1.10 | 40.1±12.8* |
| | 5 W .AT | 27 | 92.5±36.8* | 78.9 ± 50.2 | 178.8±112.3*△ | $32.89 \pm 6.30 *$ | 2.46±0.85*△ | 46.1±16.3* |
| Control | BT | 30 | 489.7 ± 153.7 | 476.9 ± 134.3 | 287.9 ± 114.9 | 31.14 ± 6.83 | 2.52 ± 0.97 | 40.2 ± 16.2 |
| | 1W.AT | 30 | 407.6 ± 134.9 | 329.7 ± 107.6 | 392.4 ± 114.4 | 27.83 ± 4.04 | 2.02 ± 0.58 | 34.6 ± 12.9 |
| | 3W.AT | 26 | 352.6±86.3△ | $231.9\pm96.2 \triangle$ | 381.7±230.4 | 26.95 ± 6.80 | 1.56 ± 0.70 | 29.3 ± 5.6 |
| | 5W.AT | 23 | 138.7 ± 51.6 | 96.7±68.4 | 246.4±120.5 | 28.12 ± 6.90 | 1.89±0.87 | 37.2 ± 9.6 |

Table 1. Changes of Biochemical Criteria before and after treatment $(\bar{x} \pm s)$

Note: BT: before treatment; AT: after treatment; * P < 0.05, compared with the control group; $\Delta P < 0.05$, compared with the same group before treatment

former one than in the latter two statistically (P < 0.01).

After 2 weeks of treatment, levels of TNF- α and ET all lowered significantly, the decrement was significantly higher in the treated group than that in the control group, resulting in a significant difference of the levels between the two groups after treatment (P < 0.05 or P < 0.01).

Table 2. Changes of TNF- α and ET Levels $(\bar{x} \pm s)$

| Group | n | TNF-α(µg/L) | ET(Eu/ml) |
|------------|-------|------------------------------|-------------------|
| Treated LS | BT 9 | 136.9±38.0 * | 0.254 ± 0.039 |
| | AT | 39.1±13.6 🗠 | 0.064±0.017☆ |
| CHB | BT 12 | 193.3±34.0* | 0.380 ± 0.036 |
| | AT | 58.4±14.3 ^{AA} | 0.109±0.014∞ |
| SCH | BT 9 | 491.4 ± 132.2 | 0.726 ± 0.190 |
| | AT | $113.2 \pm 25.5^{\triangle}$ | 0.214±0.020∽ |
| Control LS | BT 10 | 137.3±36.0* | 0.272 ± 0.056 |
| | AT | 61.7 ± 14.2 | 0.103 ± 0.035 |
| CHB | BT 11 | 203.4±36.0* | 0.366 ± 0.045 |
| | AT | 104.1 ± 22.0 | 0.145 ± 0.015 |
| SCH | BT 9 | 460.7 ± 104.0 | 0.717 ± 0.173 |
| - | AT | 167.1 ± 51.5 | 0.296 ± 0.044 |

Notes: BT:before treatment; AT:after treatment; * P < 0.01, compared with the SCH subgroup in the same group; $\triangle P < 0.05$, $\triangle \triangle P < 0.01$, compared with the control group with corresponding diagnosis

DISCUSSION

It has been demonstrated that the levels of serum ET and TNF- α increased obvioulsly in direct proportion to the severity of the disease^(5,6). ET is a kind of lipid polysaccharide in the cell of Gram's negative bacteria, consisting mainly of lactone A. It could promote the releasing of cell medium, initiate the process of endogenous coagulation to cause functional disorders of organs. Endotoxinemia could injure directly the liver cell, bile duct and cardiovascular system to induce hepatic coma, hepato-nephrotic syndrome, disseminated intravascular coagulation, toxic intestine paralysis, etc. It could also excite the macrophage to produce large amount of TNF- α , induce serious inflammatory response of organism and lead to multiple organ fail $ure^{(7)}$. The activity of TNF- α was very low in normal condition, but certain pathological condition could get large amount of $TNF-\alpha$ released to the local tissue and body fluid, or cause it imbalanced with other cytokines, so as to mediate the injury of some pathogens on the body⁽⁸⁾. So TNF- α is regarded as the key medium of inflammation promoting agent in the physio-pathological process of endotoxin induced hepatopathy⁽⁹⁾.

TCM holds that severe hepatopathy was mostly caused by accumulation of evil Damp, Heat and Phlegm, and congregating with stasis and toxin, so as to bring on the invasion by excessive Toxic-Heat of Liver, Gallbladder, Spleen and Stomach, or even penetration into Heart-sac, manifesting mainly as coma, bleeding, ascites and jaundice clinically. Active treatment of severe hepatopathy with integrated traditional Chinese and western medicine, according to the characteristics of its main pathogenesis and starting from Syndrome Differentiation of through Wei-Qi-Ying-Blood(卫气营血) series or Zang-Fu(脏 腑), has won certain effects⁽¹⁰⁾.

CCD had been used by YANG DG, et al⁽¹⁾ to treat patients of severe hepatopathy with heavy jaundice and protracted ascites and good effect had been gained. Experimental study⁽²⁾ showed that CCD has certain preventive effect on acute liver failure, which could significantly reduce the contents of ALT, AST, TB, ET and TNF- α in serum, relieve the pathological injure of liver tissue and inhibit hepatocyte apoptosis. Of the drugs in CCD, red peony root could activate blood circulation to remove stasis, subside swelling and cool blood; rhubarb could clear Heat, detoxify and activate blood circulation to remove stasis; and immature bitter orange, magnolia bark and Glauber's salt are used as auxiliaries for loosening Middle-jiao (中焦), regulating Qi, dissipating accumulation and removing chock. As a whole, it mainly acts to dissolve stasis, drain bile, purge and remove Heat and toxic substances. Modern pharmacological study showed⁽¹¹⁾ that red peony root has effects of improving microcirculation in liver, antagonizing lipid peroxidation, enhancing phagocytosis of reticulo-endothelial system, and could advance the repairing of injury and regeneration of liver cells in patients; rhubarb could improve the secretion and excretion of bile, eliminate the inflammation of hepatocytes and stasis of bile, showing anti-bacterial and anti-viral effects. The combination of these drugs could inhibit the super-sensitive response, dispel toxin and pathogenic germs and reduce the enterohepatic circulation of cholic acid. It was illustrated in this clinical observation that application of CCD upon the basis of comprehensive treatment in treating patients of severe hepatopathy could improve ALT, AST, TB, PTA, TC and ALB 2 weeks earlier than that in the control group, and cause obvious lowering of the abnormally heightened levels of TNF- α and ET, and the lowering was significantly different from the results in the control group, suggesting that CCD is helpful in improving liver function.

But the detail of its mechanism remains to be further explored.

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