Screening of drugs for Jiu Dan syndrome animal model

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Abstract: Objective: To establish an experimental animal model that conforms to the traditional Chinese medicine (TCM) syndrome of Jiu Dan. Methods: Based on the historical discourses of TCM practitioners on the Jiu Dan syndrome, the study deeply analyzed the mechanisms behind the formation of Jiu Dan syndrome. Taking into full consideration the clinical diagnostic basis of the TCM syndrome animal model, which includes symptoms, etiology, treatment, related factors, and objective indicators, a high-fat diet combined with free access to ethanol was used to induce spleen and stomach deficiency in rats, followed by the induction of liver injury using carbon tetrachloride, aminogalactose, and α-naphthylisothiocyanate. By observing the general biological status and biochemical indicators of the rats, the study screened the three chemical modeling drugs, carbon tetrachloride, aminogalactose, and α-naphthylisothiocyanate, to determine the modeling factor combination that could simulate the formation mechanism of the Jiu Dan syndrome, and established an animal model that meets the characteristics of the Jiu Dan syndrome. Conclusion: Ethanol and α-naphthylisothiocyanate were selected as the modeling drugs for the TCM Jiu Dan syndrome animal model.

Keywords: TCM Jiu Dan Syndrome, Animal Model, Ethanol, Carbon Tetrachloride, Aminogalactose, α-Naphthylisothiocyanate

1. Introduction

The discourse on traditional Chinese medicine (TCM) Jiu Dan syndrome first appeared in Zhang Zhongjing's "Treatise on Cold Pathogenic and Miscellaneous Diseases". Building upon the previous discussions, Zhang Zhongjing summarized the etiology, clinical manifestations, disease progression, prognosis, and treatment of Jiu Dan based on his clinical experience [1]. Since ancient times, TCM has had a profound discussion on the causes of diseases: "When the body's vital energy is strong, pathogens cannot invade. Wherever pathogens gather, the body's energy must be deficient." Excessive alcohol consumption is the root cause of Jiu Dan. Those who are fond of alcohol often have irregular diets, overeat or under-eat, and consume fatty and sweet foods, leading to a weak spleen and stomach, which is the internal factor for the development of Jiu Dan. External pathogenic invasions, gallbladder heat, and overflow of fluids that soak the skin eventually lead to jaundice [2-3]. The replication of TCM syndrome animal models should fully consider five aspects of clinical diagnosis: symptoms, etiology, treatment, related factors, and objective indicators [4-6]. This study, through an in-depth analysis of the mechanism of Jiu Dan syndrome formation, starts from the clinical diagnostic basis of TCM Jiu Dan syndrome, combines the modeling factors that simulate the pathogenesis of Jiu Dan syndrome, and establishes an animal model that conforms to the characteristics of the etiology and pathogenesis of Jiu Dan syndrome.

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2. Materials and methods

2.1. Experimental materials

Refined lard; carbon tetrachloride; aminogalactose; α-naphthylisothiocyanate; anhydrous ethanol; pentobarbital sodium; olive oil; alanine aminotransferase assay kit; aspartate aminotransferase assay kit; alkaline phosphatase assay kit; total bilirubin assay kit; direct bilirubin assay kit; interleukin-2 assay kit; cyclic adenosine monophosphate assay kit; cyclic guanosine monophosphate assay kit. Male Wistar rats, body weight (180±20) g.

2.2. Experimental methods

2.2.1. Preparation of carbon tetrachloride reagent

Dissolve 2mL of carbon tetrachloride in 8 mL of olive oil to prepare a carbon tetrachloride-olive oil solution with a concentration of 20%. [7]

2.2.2. Preparation of aminogalactose reagent

Dissolve 2grams of aminogalactose in 20mL of physiological saline to prepare an aminogalactose solution with a concentration of 100 g/L. [8-9]

2.2.3. Preparation of α-Naphthylisothiocyanate olive oil solution reagent

Dissolve 3.2g of α-naphthol isothiocyanate in 80mL of olive oil to prepare a test solution of α-naphthol isothiocyanate in olive oil at 4% concentration. [10]

2.2.4. Preparation of ethanol solution reagent

Prepare an aqueous solution of ethanol at 6% (v/v) concentration. [11]

2.2.5. Grouping and drug administration

Seventy-five Wistar rats were randomly divided into four groups: the control group, the ethanol-carbon tetrachloride group, the ethanol-aminogalactose group, and the ethanol-α-naphthol isothiocyanate group. Control group: free diet and water for 28 days. Ethanol-carbon tetrachloride group: the rats' drinking water was replaced with a 6% (v/v) ethanol aqueous solution as the sole source of drinking water, with free access to water 24 hours a day and free feeding for 28 days [12]. Daily gavage administration of refined lard at 0.5 ml/200g body weight [13], for 28 consecutive days. On the 28th day, the rats were given a 20% carbon tetrachloride-olive oil solution by gavage at a dose of 1.0 ml/kg body weight. Ethanol-aminogalactose group: the rats' drinking water was replaced with a 6% (v/v) ethanol aqueous solution as the sole source of drinking water, with free access to water 24 hours a day and free feeding for 28 days. Daily gavage administration of refined lard at 0.5 mg/200g body weight, for 28 consecutive days. On the 28th day, aminogalactose was administered intraperitoneally at a dose of 600 mg/kg body weight. Ethanol-α-naphthol isothiocyanate group: the rats' drinking water was replaced with a 6% (v/v) ethanol aqueous solution as the sole source of drinking water, with free access to water 24 hours a day and free feeding for 28 days. Daily gavage administration of refined lard at 0.5 ml/200g body weight, for 28 consecutive days. On the 26th day, the rats were given a 4% α-naphthol isothiocyanate solution by gavage at a dose of 7.5 mg/100g body weight, and on the 27th day, they were given the same solution at a dose of 2.5 mg/100g body weight [14]. All groups of animals were weighed and then sampled on the 29th day.

2.3. Statistical analysis

All data are expressed as ($x \pm s$) and were analyzed using SPSS 17.0 software for one-way ANOVA. A P-value of less than 0.05 was considered to indicate statistical significance.

3. Results

3.1. Observation of general biological status in rats

Rats on a high-fat diet with free access to ethanol exhibited symptoms of spleen deficiency such as diarrhea, curling up, lethargy, and reduced activity. After administration of carbon tetrachloride,

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aminogalactose, and α-naphthol isothiocyanate, rats in each model group exhibited symptoms such as piloerection, hunched back, and dry and unkempt fur. The symptoms were most pronounced in the ethanol-α-naphthol isothiocyanate group, with a significant increase in urine output and the urine appeared dark yellow.

3.2. IL-2 and cAMP/cGMP ratio in serum

Compared to the normal control group, there was a significant increase in serum interleukin-2 levels in the ethanol-carbon tetrachloride group, ethanol-aminogalactose group, and ethanol-α-naphthol isothiocyanate group, with a significant difference ($P<0.01$), indicating that the immune function of the body was suppressed, with the greatest increase in interleukin-2 levels in the serum of the ethanol-α-naphthol isothiocyanate group. Compared to the normal control group, the ratio of cyclic adenosine monophosphate to cyclic guanosine monophosphate in the serum of the ethanol-carbon tetrachloride group, ethanol-aminogalactose group, and ethanol-α-naphthol isothiocyanate group was significantly reduced, with a significant difference ($P<0.01$), and the ethanol-α-naphthol isothiocyanate group showed the greatest reduction. See Table 1.

Table 1 The Impact of Ethanol Combined with Different Hepatotoxic Drugs on Immunological Indices in Rats (n=15, x ± s)

Group	IL-2(u mol/L)	cAMP/cGMP
Control Group	2.25±0.197	5.35±0.099
Ethanol-Carbon Tetrachloride Group	2.59±0.035	1.54±0.033*
Ethanol-Aminogalactose Group	2.63±0.027*	1.55±0.025*
Ethanol-α-Naphthylisothiocyanate Group	2.81±0.165*	1.27±0.163*

3.3. ALT, AST, and ALP activities in serum

Compared to the normal control group, the activity of Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), and Alkaline Phosphatase (ALP) in the serum of the Ethanol-Carbon Tetrachloride Group, Ethanol-Aminogalactose Group, and Ethanol-α-Naphthylisothiocyanate Group was significantly increased, showing a significant difference ($P<0.01$), indicating liver damage in rats. Among these, the Ethanol-Carbon Tetrachloride Group showed the greatest increase in AST values, and the Ethanol-α-Naphthylisothiocyanate Group showed the greatest increase in ALT and ALP values (see Table 2).

3.4. Direct and Total bilirubin levels in serum

Compared to the normal control group, there was a significant increase in the levels of direct bilirubin and total bilirubin in the serum of the Ethanol-Carbon Tetrachloride Group and the Ethanol-α-Naphthylisothiocyanate Group, with a statistically significant difference ($P<0.01$), indicating cholestatic liver injury. Among them, the Ethanol-α-Naphthylisothiocyanate Group showed the greatest increase in the levels of direct bilirubin and total bilirubin (see Table 2).

Table 2 The Impact of Ethanol Combined with Different Hepatotoxic Drugs on Various Clinical Biochemical Indices in Rats (n=15, x ± s)

Group	AST(U/L)	ALT(U/L)	ALP(U/L)	DBIL (μ mol/L)	TBIL (μ mol/L)
Control Group	91.69±12.17	36.13±6.59	124.12±23.82	0.32±0.22	1.46±0.37
Ethanol-Carbon Tetrachloride Group	398.29±50.38*	272.07±92.29*	216.44±51.39*	44.20±5.61*	58.29±6.66*
Ethanol-Aminogalactose Group	267.72±54.22*	113.44±46.18*	212.73±53.85*	4.68±0.94*	4.69±0.63*

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Ethanol-α-Naphthylisothiocyanate Group	328.58±66.75*	308.87±63.23*	262.44±36.35*	92.94±16.64*	120.22±11.57*

4. Discussion

The experimental results indicate that the activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) in the serum of the Ethanol-Carbon Tetrachloride Group, Ethanol-Aminogalactose Group, and Ethanol-α-Naphthylisothiocyanate Group were significantly increased compared to the normal control group, with a statistically significant difference ($P<0.01$), indicating liver damage in the rats. Among these, the Ethanol-Carbon Tetrachloride Group showed the greatest increase in AST values, while the Ethanol-α-Naphthylisothiocyanate Group showed the greatest increase in ALT and ALP values. The Ethanol-Carbon Tetrachloride Group and the Ethanol-α-Naphthylisothiocyanate Group had significantly increased levels of direct bilirubin and total bilirubin in the serum, with a statistically significant difference ($P<0.01$) compared to the normal control group, indicating cholestasis, with the Ethanol-α-Naphthylisothiocyanate Group showing the greatest increase in direct bilirubin and total bilirubin levels. The levels of interleukin-2 (IL-2) in the serum of the Ethanol-Carbon Tetrachloride Group, Ethanol-Aminogalactose Group, and Ethanol-α-Naphthylisothiocyanate Group were significantly elevated, with a statistically significant difference ($P<0.01$) compared to the normal control group, indicating suppression of the immune system, with the greatest increase in IL-2 levels observed in the Ethanol-α-Naphthylisothiocyanate Group. The ratios of cyclic adenosine monophosphate (cAMP) to cyclic guanosine monophosphate (cGMP) in the serum of the Ethanol-Carbon Tetrachloride Group, Ethanol-Aminogalactose Group, and Ethanol-α-Naphthylisothiocyanate Group were significantly reduced, with a statistically significant difference ($P<0.01$) compared to the normal control group, with the greatest reduction observed in the Ethanol-α-Naphthylisothiocyanate Group.

The effectiveness of traditional Chinese medicine (TCM) syndrome animal models directly affects the evaluation of the efficacy of Chinese herbal medicine and clinical therapeutic effects. Although scholars have done a great deal of work in the study of TCM syndrome animal models, the replicated animal models are mostly physiological and pathological models, which cannot essentially reflect the formation, development, and progression of TCM syndromes [15]. This study, based on the historical discourses of medical practitioners on TCM Jiu Dan syndrome as a blueprint, deeply analyzes the mechanism of formation of TCM Jiu Dan syndrome. On the basis of fully considering the five aspects of clinical diagnostic basis of TCM syndrome animal models, which include symptoms, etiology, treatment, related factors, and objective indicators, a method of high-fat diet combined with free access to ethanol was used to induce spleen and stomach deficiency in rats, followed by the induction of liver injury in rats with carbon tetrachloride, aminogalactose, and α-naphthol isothiocyanate. By observing the general biological status and biochemical indicators of the rats, the three chemical modeling drugs, carbon tetrachloride, aminogalactose, and α-naphthol isothiocyanate, were screened to determine the modeling factor combination that could simulate the mechanism of formation of TCM Jiu Dan syndrome, thereby establishing an animal model that conforms to the characteristics of the Jiu Dan syndrome.

5. Conclusion

In this study, we successfully established an experimental animal model that simulates the TCM Jiu Dan syndrome, providing an important experimental basis for in-depth study of the pathological mechanisms of TCM Jiu Dan syndrome and the exploration of treatments with Chinese herbal medicine. By comprehensively considering the etiology, symptoms, treatment principles, and related factors of TCM Jiu Dan syndrome, this study employed a combination of a high-fat diet with ethanol intake, along with the chemical inducers carbon tetrachloride, aminogalactose, and α-naphthol isothiocyanate, to conduct a series of carefully designed experimental operations on rats. The experimental results showed that the ethanol-α-naphthol isothiocyanate group demonstrated the most pronounced effects in simulating multiple

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biochemical indicators and biological states of Jiu Dan syndrome, thereby validating the effectiveness of the model.

The conclusion of this study emphasizes the necessity of considering multiple factors when constructing TCM syndrome animal models to ensure that the models can truly reflect the characteristics of TCM syndromes. In addition, through this study, we have further understood the intrinsic connections between excessive alcohol consumption, spleen and stomach deficiency, liver damage, and their roles in the pathogenesis of Jiu Dan syndrome. This discovery not only enriches the theoretical system of TCM Jiu Dan syndrome but also provides new perspectives and methods for clinical treatment. In the future, we will continue to optimize and improve this model, with the expectation of playing a greater role in the research and clinical application of traditional Chinese medicine.

6. References

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