

Research on the evaluation indexes of animal model of liver-fire hypertension syndrome

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Abstract. Objective: The screening of animal models combined with disease and syndrome is the basis and key to TCM experimental research. According to relevant reports, SHR at 14-18 weeks of age shows a stable type of hypertensive liver-heat syndrome, and the macroscopic appearance and rotation tolerance time of the rat from this experiment. Such as a number of indicators, initially clearly distinguish the relevant microscopic physical and chemical indications of the type of syndrome. Methods: eight 14-week-old SHR were selected as the model group and 8 normal WKY rats were used as the control group. The rats were observed continuously for 5 weeks. Based on the transformation of diagnostic criteria of liver-fire hyperactivity syndrome, macroscopical images of animals were collected. Systolic blood pressure (SBP) and serum angiotensin II (Ang II) and noradrenaline (NE) levels were measured by enzyme linked immunosorbent assay (ELISAA) at the age of 18 weeks. Results: compared with the control group, the systolic blood pressure and irritability of the rats in the 14-18 week old model group were obviously increased, while the rotational tolerance time was decreased. The serum Ang II NE increased significantly in the model group at the age of 18 weeks ($P < 0.05$). Conclusion Ang II NE elevation is an important experimental index for evaluating hyperactivity of liver fire in patients with hyperactivity of liver-fire at the age of 14 to 18 weeks.

1. Introduction

Syndrome differentiation is the living soul of traditional Chinese medicine theory. Only by combining disease and syndrome can we fully clarify the scientific connotation of the disease syndrome. Therefore, the selection of scientific and stable animal models combination of disease and syndrome, as a cornerstone and bridge, using modern scientific methods will provide important experimental evidence for the diagnosis of syndromes and evaluation of drug efficacy.

According to relevant literature reports, for the preparation of animal model of liver-fire hypertension syndrome, the best method is to select the spontaneous hypertensive rats whose etiology and pathogenesis are very similar to those of human hypertension to be an animal model, combining the macroscopic appearance and microscopic indexes of the rats, and to compare by the diagnosis and conversion of TCM syndromes as the basis. To screen and evaluate the disease- syndrome binding animal model of Liver-fire Hypertension Syndrome.



According to relevant literature reports, spontaneously hypertensive rats (SHR) present liver-fire hypertension syndrome during 14-18 weeks of age [1], and studies have shown that elevated Ang II and NE may be important reference indexes for hypertension of liver-fire hyperactivity [2], significantly higher than other syndromes. Therefore, in this experiment, we observed the changes of the rat with this syndrome in macroscopic appearance, systolic blood pressure, rotation tolerance time, irritability score, and microscopic indexes such as Ang II and NE. preliminary clear objective biological indicators of liver-fire hypertension syndrome.

2. Experimental Materials and Methods

2.1. Experimental Animals

8 spontaneously hypertensive rats (SHR) (purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd), SPF grade, 12-week-old, male, and 8 WKY rats (purchased from The Animal Experiment Center of Henan Province), 12-week-old, male, both of which were raised in the animal room of the Animal Experimental Center of Henan University of Traditional Chinese Medicine, were adapted for feeding for 2 weeks.

2.2. Major Instruments and Reagents

Major Instruments: Shanghai Precision Electronic Balance; Balanced Rotation Apparatus; Constant Temperature Water Bath; Japan Softron BP-98A Rat Noninvasive Tail Arterial Manometer, German Low Temperature High Speed Centrifuge, Rat Serum (AngII, NE) Enzyme-Linked Immunosorbent Assay Reagent Box from Wuhan Elabscience Biotechnology Co.,Ltd.

2.3. Animal Grouping

According to the diagnostic criteria of clinical syndromes, 8 SHR were subjected to equivalent transformation by macroscopic characterization, and syndrome attribute discrimination was performed [1, 3]. In combination with systolic blood pressure, rotation tolerance time, irritability score and macroscopic appearance, all the results were expressed as Rats with liver-fire hypertension syndrome, 8 normal WKY rats as control group.

2.4. Preparation of Materials and Samples

At the age of 18 weeks old, the rats of the two groups were fasted for one night. The next day, they were intraperitoneal injection anesthetized with 10% chloral hydrate 3.5 ml/kg, rapidly incised along the midline of abdomen and about 10ml blood was taken via the abdominal aorta. Each group of samples was placed in a vacuum blood collection tube containing sodium citrate. After standing for 1 hour, used low-temperature high-speed centrifuge (3000 rpm) for 5 minutes, taken out the serum and stored in a refrigerator at -80 °C. Preparation for indicators for testing.

3. Experimental index detection

3.1. Measurement of Systolic Blood Pressure

The systolic blood pressure of the rat was measured using the caudal artery pressurization method. The specific procedures were as follows. When the rat was awake, first, fixed the rat in a cage and then placed in a thermostat, and the temperature was preheated for about 10 minutes. The blood vessels in the tail were expanded, and the tail was fully exposed. The systolic blood pressure was measured after being clamped with a blood pressure clamp. Measurements were repeated 3 times, and the average value was taken as the blood pressure result. The measurement was made once a week.

3.2. Measurement of Rotation tolerance time

First, set the rotation speed of the JD-SH to 60 r/min. Then place the rat in the center of the rotating platform, start the power supply, and start timing until the rat falls off the rotating platform and record the time.

3.3. The Degree of Irritability Reference Rating

According to the relevant literature [3], its reference rating standards are shown in Table 1.

Table 1. Irritability level reference rating

No.	Level	Evaluation criteria and score
1	Class I	There was no obvious reaction while holding the rat's neck (score 1)
2	Class II	Screaming and jumping while holding the rat's neck (score 2)
3	Class III	Biting while holding the rat's neck or frequently fighting with other rats (score 3)

3.4. Data Analysis

The experimental data were analyzed using statistical software SPSS18.0. The variance analysis was used for the measurement data among the groups, expressed as the mean $\pm$ standard deviation ($\bar{x} \pm S$). The count data was used for the rank sum test. When $P < 0.05$, Showed that the difference was significant and statistically significant.

4. Results

4.1. The Changes of Macroscopic Appearance in Two Groups of Rats Aged 14-18 weeks

Compared with the control group, the 14-18 weeks old SHR all showed signs of liver-fire hypertension syndrome. They were excited, Likes fighting, strong resistance when grabbing. their tongue were thin and dry, and the blood vessels of the ears and claws were relatively full. their urine is less and yellow ,their stools were hard and dry. However, the normal control rats had no excitement, no obvious revolt, and no obvious change in fur color.

4.2. Changes of Systolic Blood Pressure in 14-18 Weeks Old Rats

The results in Table 2 indicate that the systolic blood pressure was significantly higher in the 14-18 weeks old model group compared with the control group, which is based on the standard of hypertension diagnosis--systolic blood pressure is 140 mmHg, and the difference is significant ($P < 0.05$).), and systolic blood pressure gradually increased with the time of feeding, and was positively correlated with time.

Table 2. Comparison of rat systolic blood pressure ($\bar{x} \pm S$) (n=8) unit: mmHg

Group	W14	W15	W16	W17	W18
The control group	124.9 $\pm$ 5.11	124.53 $\pm$ 5.19	125.42 $\pm$ 7.59	125.08 $\pm$ 5.25	125.86 $\pm$ 9.46
The model group	190.03 $\pm$ 6.1*	195.8 $\pm$ 6.01*	214.93 $\pm$ 12.02	220.40 $\pm$ 13.6*	212.75 $\pm$ 8.57*

Note: *compare with the control group, $P < 0.05$

4.3. The Changes of Irritation-Degree Scores in Two Groups Of Rats at 14-18 Weeks of Age

The results in Table 3 show that compared with the control group, the rotation tolerance time of SHR rats at 14-18 weeks of age is significantly shortened. According to the equivalent transformation of clinical diagnostic criteria, the rotation tolerance time corresponds to one of the main symptoms of liver-fire hypertension syndrome--"dizziness". The indications are one of the important criteria for distinguishing liver-fire hypertension syndrome.

Table 3. Comparison of rat irritability scores (n=8)

Group	W14	W15	W16	W17	W18
The control group	1.13 $\pm$ 0.35	1.25 $\pm$ 0.46	1.25 $\pm$ 0.71	1.13 $\pm$ 0.35	1.13 $\pm$ 0.35
The model group	2.75 $\pm$ 0.46	2.88 $\pm$ 0.35	2.88 $\pm$ 0.35	3 $\pm$ 0	3 $\pm$ 0*

Note: *compare with the control group, $P < 0.05$

4.4. Effect of Serum Ang II and NE Levels in Two Groups of Rats at the Age of 18 Weeks

Table 4 shows that compared with the control group, the levels of NE and Ang II in the serum of the model group were significantly increased at the age of 18 weeks ($P < 0.05$).

Table 4. Comparison of NE and Ang II levels in the two groups of rats at the age of 18 weeks ($\bar{x} \pm S$)($n=8$)

Group	Unit: mmol/l	
	NE	Ang II
The control group	1.39 $\pm$ 0.14	77.98 $\pm$ 4.90
The model group	3.66 $\pm$ 0.29*	147.65 $\pm$ 5.83*

Note: *compare with the control group, $P < 0.05$

5. Discussion

According to the theory of traditional Chinese medicine, uncomfortable Feeling, anxiety and anger can induce visceral blood disorders, leading to the occurrence of various diseases. Among them, emotions are most likely to affect the catharsis of the liver, and if the catharsis is abnormal, the running of the blood is abnormal, the blood is depressed, and the depress of the blood will becoming fire, and the fire is changed [4]. The blood is in disorder, and can cause dizziness, headache. Therefore, liver-fire hypertension syndrome is a more common clinical syndrome of hypertension [5, 6, 7].

A large number of studies have found that high blood pressure is often accompanied by increased activity of the peripheral sympathetic nervous system [8, 9]. NE acts as a neurotransmitter, which can cause vasoconstriction of various organs of the whole body, causing an increase in peripheral vascular resistance, resulting in an increase in blood pressure and may lead to Vascular fibrosis [10]. Ang II is one of the core substances in the renin-angiotensin-aldosterone system. Ang II has the effect of directly constricting blood vessels, and it can accelerate the process of hypertension by a variety of ways [11, 12].

The results of this experiment shows that based on the equivalent conversion of the syndrome criteria of traditional Chinese medicine, 14-18 weeks old SHR is a stable phase of liver-fire hypertension syndrome, and this syndrome is related to certain vasoactive substances, Among them, AngII and NE can be used as experimental references for the diagnosis and therapeutic evaluation of liver-fire hypertension syndrome.

6. References

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