Establishment and Evaluation of a Rat Model of Hypertension with Liver Prosperity and Phlegm Obstruction Syndrome

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ABSTRACT Objective: To establish and evaluate the model of spontaneity hypertension rat with liver prosperity and phlegm obstruction syndrome. Methods: To choose spontaneity hypertension rat and adopt the method of slow irritation and fat-rich diet to establish complex syndrome of liver prosperity and phlegm obstruction, by observing the dynamic changes of the temperament and weight, blood pressure, blood fat and angiotensin Ⅱ, comprehensively evaluated the rats with liver prosperity and phlegm obstruction syndrome. Results: Dynamic changes of the temperament and weight, blood pressure, blood fat and angiotensin Ⅱ in the model group had a significant difference compared with controls ($p<0.05$), conformed to the characteristic of liver prosperity and phlegm obstruction syndrome. Conclusions: The model of spontaneity hypertension rat with liver prosperity and phlegm obstruction syndrome could be established by means of slow irritation and fat-rich diet.

Key words: Liver-yang hyperactivity; Phlegm obstruction; Spontaneously hypertensive rat (SHR); Animal model

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Introduction

There are records of high blood pressure disease in Chinese medicine, and the general symptom belongs to vertigo, liver-yang, liver wind [1] and so on disease category. Different scholars have their own criterions about the definition of hypertension in Chinese medicine, although the hypertensive standard of diagnosis is different, it is easy to find the basic syndrome of high blood pressure is liver-yang hyperactivity [2-3] or phlegm obstruction [4-5]. The clinic practice further finds hypertension has many complex syndromes, but the liver prosperity and phlegm obstruction is one of the most common complex syndrome [6]. In order to further study the complex syndrome, this article adopts the way of slow irritation and fat-rich diet to establish the model of SHR with liver prosperity and phlegm obstruction syndrome.

1 Materials and methods

1.1 Experimental animal

Sixteen male SHR, weighted (210.73± 19.43) g, were purchased form Experimental Animal Center of Beijing Vital River Laboratories.

1.2 Experimental instruments and reagents

Biochemical automatic analyzer (Hitachi 7150 type),RBP Rat tail artery pressure gauge, angiotensin Ⅱ kit, Total Cholesterol kit, TG kit, the reagents were offered by Shanghai Jin ma biological technology company.

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1.3 Animal high-fat forage

High-fat forage: lard oil 5%, yolk powder 10%, sodium cholate0.5%, cholesterol 2% general feed 82.5%,the forages were offered by Experimental Animal Center of Beijing Vital River.

1.4 Method of establishing liver prosperity and phlegm obstruction model

Sixteen healthy SHR were randomly divided into the model group and the blank control. The control group was provided general forage, and the model group was offered by a slow irritation to establish the liver-yang hyperactivity model [7], which meant binding double hind legs of the rats, and both were hang upside down in the cage in order to obviously irritate, they manifest squawk or bite. The first irritation time was 20 minutes, once per day, and then added 10 minutes every week, meanwhile fed the fat-rich diet to establish the phlegm obstruction model [8-9], the total time was four weeks. At the end of the fourth week, by observing dynamic changes of the temperament and weight, blood pressure, blood fat, referring to the evaluation score sheet of liver-yang hyperactivity[10] to determine higher twelve score was liver-yang hyperactivity, at the same time enucleated eyeball and collected blood to detect blood biochemical indicators, then confirmed to have established the model of SHR with liver prosperity and phlegm obstruction syndrome.

1.5 Methods of evaluating liver prosperity and phlegm obstruction syndrome

1.5.1 Behavioral manifestation By observing temperament change of the rats, whether the color of eye conjunctiva deepened, and turned red .There were three grades according to the irritability level, Ⅰ level meant that when grasping the neck of the rats, they had no significant response; Ⅱ level meant that when grasping the neck of the rats, they scream, startled ; Ⅲ level meant that when grasping the neck of the rats, they bit or frequently fought in

the same cage[11].

1.5.2 Body weight measurement Weight of the rats was measured one time before modeled and at the end of the fourth week.

1.5.3 Blood pressure measurement [12] The tail artery systolic blood pressure of the rats was measured once per day for three consecutive days before modeled and at the end of fourth week.

1.5.4 Rotation time measurement [13] Put the rats on the rotary table, and rotated them at 45r/min,if the rats could not drop after being rotated for 2 minutes, then stopped the experiment.

1.5.5 Blood biochemical detection At the end of the fourth week ,all the rats were forbid food for 12 hours to detect Angiotensin Ⅱ, Cholesterol (TC), Triglyceride (TG), and measured weight and blood pressure, meanwhile 4ml blood was collected form eye artery, and EDTA-Nat was rapidly added into anti-freeing tube, then the blood was centrifuged for10minutes at 1000rpm to separate the serum and then stored at -20℃. Changes of Angiotensin Ⅱ, TC, TG could be detected with Elisa according to the instruction of reagents.

1.5.6 Statistical processing method All data were expressed as mean ± standard deviation, SPSS17.0 software was used to make t-test analysis.

2 Experimental results

2.1 Animal behavior observation results

Blank healthy rats acted flexibly, quickly responded to the wink of their eyes, and had a sensitive reaction ;the performance of model group was agitated, irritable, at the same time they fought each other, the color of eye conjunctiva deepened and turned red, and the model group had a significant difference form the control (P< 0.05). (Table 1).

Table 1 Behavior observation($\bar{X}$± S)

Group	n	Temperament change			Conjunctival suffusion
		Ⅰ grade	Ⅱ grade	Ⅲgrade	
Blank control	8	2 (25%)	0	0	0
Model group	8	1 (12.5%)	3a (37.5%)	4a (50%)	7a (87.5%)

Note :A Compared with blank, P< 0.05

2.2 Rotation time measurement results

Before modeled, rotation time of model group was 116.59± 1.82 min, while after modeled the rotation time shortened, it was 40.65± 3.37 min, which was significantly different with the time before modeled and the time of controls (P<0.05).

Table 2 Rotation time before and after modeled($\bar{X}$± S)

Group	n	Before modeled	After modeled
Model group	8	116.59± 1.82	40.65± 3.37a
Blank control	8	115.31± 1.99	115.40± 1.78

Note: After modeled, a Compared with the blank control, P<0.05

2.3 Weight and blood pressure measurement results

Before modeled, weight and blood pressure of the model were 201.15± 11.06 g, 160.16± 2.04 mmHg respectively, while after modeled ,weight and blood pressure had a significant increase, they were 356.95± 24.46 g, 188.63± 4.3 mmHg respectively, which were significantly different with those before modeled and those of controls (P<0.05).

2.4 Blood biochemical test results

After modeled, the levels of Blood fat and Angiotensin Ⅱ in the model group were2.03 ± 0.11, 2.15 ± 0.11, 371.7 ± 18.6, mmoloL^{-1} respectively, which were significantly higher than controls compared with controls (P<0.05).

Table 3 Weight and blood pressure before and after modeled($\bar{X}$± S)

Group	n	Before modeled		After modeled	
		Weight(g)	Blood pressure(mmHg)	Weight(g)	Blood pressure(mmHg)
Model group	8	201.15± 11.06	160.16± 2.04	356.95± 24.46a▲	188.63± 4.3a▲
Blank control	8	203.10± 13.88	157.69± 2.35	251.96± 11.69	160.54± 3.3

Note: A Compared with before modeled, P<0.05,▲Compared with Blank control, P<0.05

Table 4 The levels of Blood fat (mmoloL^{-1}) and Angiotensin Ⅱ (mmoloL^{-1}) after modeled($\bar{X}\pm$ S)

Group	n	TG	TC	Ang Ⅱ
Model group	8	2.03± 0.11a	2.15± 0.11a	371.7± 18.6a
Blank control	8	1.24± 0.66	1.22± 0.46	151.6± 5.5

Note: After modeled, a Compared with the blank control, P<0.05

3 Discussion

This experiment adopted the method of slow irritation and fat-rich diet to establish the model of SHR with liver prosperity and phlegm obstruction syndrome, and evaluated the model through the behavior manifestation and objective indicators.

The behavior manifestation characteristic was showed by changes of the temperament, rotation time and the color of eye conjunctiva, while objective indicators were mainly reflected by changes of blood pressure, weight, blood fat, angiotensin Ⅱ.

The results showed two groups had no obvious difference before experiment, after four weeks, the model group became easily agitated and irritable, and the color of eye conjunctiva deepened and the rotation time shortened, most these changes belonged to Ⅱ、Ⅲ level, at the same time blood pressure of the model group increased and had a significant difference compared with controls, and the model rats had the characteristic of liver-yang hyperactivity syndrome. Furthermore, the obvious difference of angiotensin Ⅱ compared with the control group also confirmed the model rats had character of liver-yang hyperactivity syndrome. Research showed that experimental animals possessing liver- yang kept higher renin activity and angiotensin Ⅱ than normal. Higher angiotensin Ⅱ was one of the most important objective factors in liver-yang hyperactivity syndrome [14].

Chinese Traditional Medicine classified obesity as "flesh man" or "fat man" and thought the reason was "phlegm" and "dampness". Fat man had more "phlegm" and "dampness" than normal. The fact that weight of model group increased significantly compared with control group indicated the model rats had phlegm-dampness, and the fact that the levels of blood fat increased significantly compared with the control also indicated the model rats had characteristic of phlegm-dampness. Study [15] also showed the rats fed on fat-rich diet not only had characteristic of phlegm-dampness also had the characteristic of "fat, fatty liver, hyperlipidemia , insulin resistance" .

Therefore, a conclusion could be drawn that by slow irritation and fat-rich diet could establish the model of SHR with liver prosperity and phlegm obstruction syndrome.

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肝旺痰阻型高血压大鼠模型的建立和评价研究

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摘要 目的:建立和评价肝旺痰阻型高血压大鼠模型。方法:采用自发性高血压大鼠,以长期激怒联合高质饮食法建立肝旺痰阻的复合证候。通过观察大鼠性情动态的变化及体重、血压及血脂和血管紧张素Ⅱ的变化,对高血压大鼠肝旺痰阻证型进行综合评价。结果:模型组大鼠在性情动态及体重、血压、血脂和血管紧张素Ⅱ等方面均与对照组有较大差异($P<0.05$),符合了中医肝旺痰阻证型的表现。结论:采用自发性高血压大鼠,以长期激怒联合高质饮食法,可建立肝旺痰阻型高血压大鼠动物模型。

关键词 肝旺痰阻;自发性高血压大鼠;动物模型

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