Basic Investigations

Effect of Zibu Shenjing Fang (滋补肾精方) on Growth and Development of the Mouse with Kidney-essence Insufficiency and Study on the Mechanism

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Objective: To investigate effects of Zibu Shenjing Fang (滋补肾精方) on growth and development of the mouse with insufficiency of kidney-essence and the mechanism.

Methods: Total 50 mice were randomly divided into a normal group, a model group, a Jingui Shenqi Wan (金匮肾气丸) group, a Zibu Shenjing Fang high dose group and a Zibu Shenjing Fang low dose group, 10 mice in each group. The kidney-essence insufficiency mouse model was established by use of threat-injuring the kidney combined with over-fatigue. At the same time of modeling, the mice in the model group were intragastrically administrated with saline 20 mL·kg⁻¹·d⁻¹, in the Jingui Shenqi Wan group with suspension of the Jingui Shenqi Wan 2.7 g·kg⁻¹·d⁻¹, in the Zibu Shenjing Fang high dose group with Zibu Shenjing Fang 20 g·kg⁻¹·d⁻¹ and in the Zibu Shenjing Fang low dose group with Zibu Shenjing Fang 10 g·kg⁻¹·d⁻¹, for 21 consecutive days. The general state was observed, the body weight was weighted, and serum growth hormone (GH) and insulin-like growth factor-1 (IGF-1) contents were detected.

Results: Compared with model group, Zibu Shenjing Fang groups and Jingui Shenqi Wan group could improve manifestation of the mouse with kidney-essence insufficiency, increase body weight of the mouse and serum GH and IGF-1 contents, especially in the high dose group.

Conclusion: Zibu Shenjing Fang gives play to the function of tonifying the kidney and replenishing essence through regulating GH and IGF-1 levels, so as to influence growth and development of the mouse.

Keywords: Zibu Shenjing Fang; kidney-essence insufficiency syndrome; mechanism

Traditional Chinese Medicine holds that the kidney dominates store of essence and has an important regulatory action on growth and development of the organism. Therefore, clinically delay of development, senilism and other diseases all are closely related with insufficiency of kidney-essence. In order to study on the essence of the kidney being in charge of store of essence and kidney-essence insufficiency insufficiency syndrome, as well as the mechanism of Chinese drugs for tonifying the kidney and replenishing essence in improving growth and development of the organism, in the present study, the kidney-essence insufficiency mouse model was established with cat threatening mouse combined with exhausting swimming, and effects of Zibu Shenjing Fang (滋补肾精方) on the general state, the body weight, and serum growth hormone (GH) and insulin-like growth factor-1 (IGF-1) contents were observed in the kidney-essence insufficiency model mouse.

MATERIALS AND METHODS

Animals
Healthy Kunming strain SPF mice, male, body weight 18–22 g, were supplied by Medical Experimental Center, Lanzhou University (Certificate of quality No: SKXX 2010-00014), and one male cat (Flis catus).

Drugs
Zibu Shenjing Fang was composed of 10 Chinese medical herbs, including Shu Di Huang (Radix Rehmanniae Preparata), Shan Yu Rou (sarcocarp of Fructus Corni), Ba Ji Tian (Radix Morindae Officinalis), Hong Qi (Radix Hedysari), Dang Gui (Radix Angelicae Sinensis), etc., which were immerged in 500 mL cool water for 30 min, and heated with strong fire till boiling and decocted with mild fire for 30 min, and then filtered. The dregs of decoction were added with 500 mL water and decocted for another 30 min. Both filtrates were poured together and concentrated with water bath as a solution of crude drugs 2 g·mL⁻¹ and kept at a refrigerator at 4 °C. Jingui Shenqi Pills (金匮肾气丸) made by Pharmaceutical Factor, Beijing Tonrentang Science and Technique Development Co. LTD were prepared as 0.2 g·mL⁻¹ suspension with distilled water.

Main Reagents and Equipments
Mouse IGF-1 and mouse GH enzyme linked
immunosorbent assay (ELISA) kits were purchased from Kehao Bioengineering Co. LTD (batch No: 201004). Enzyme linked immunosorbent detector, KDC-2046 low temperature large volume centrifuge (Zhongjia Photoelectric Instruments Branch Company, General Company of Science and Technique Industry and Commerce, China University of Science and Technology), swimming jar, thermostat water box, electric balance, needle for intragastrical perfusion.

Modeling and Grouping
The mice were divided into 5 groups, a normal group (NG), a model group (MG), a Jingui Shenqi Wan group (JGG), a Zibu Shenjing Fang high dose group (HDG) and a Zibu Shenjing Fang low dose group (LDG) by random number table, 10 mice in each group. Except the NG, the mice in other groups were placed under the cat cage with only a network separated from the cat. With such way, the cat threatened the mice for 24 h, and one living mouse each was taken to feed the cat for public exposure in both the morning and evening each day. At 2 o'clock in afternoon each day, the mice were forced to swim in a plastic jar with 25 cm in depth and 10,000 mL in volume at 25°C till sank due to weakness and then were taken out, inducing over fatigue, for 21 consecutive days.1

Administration
At the same time of modeling, the mice in the MG were intragastrically administrated with saline 20 mL kg⁻¹·d⁻¹ each day, in the JGG with Jingui Shenqi Wan suspension 2.7 g·kg⁻¹·d⁻¹, in the HDG with Zibu Shenjing Fang 20 g·kg⁻¹·d⁻¹ and in the LDG with Zibu Shenjing Fang 10 g·kg⁻¹·d⁻¹ each day, for 21 consecutive days.

Observation Indexes
General state of the mice: Skin and hair, expression, activity, drinking, feeding and coordination in intragastrical perfusion, and the body weight were observed before and after experiment.

Determination of serum GH, IGF-1 contents: 30 min observed before and after experiment.

Results of Zibu Shenjing Fang on the body weight of the mouse

Table 1. Results of Zibu Shenjing Fang on the body weight of the mouse (x ± s, n=10)

<table>
<thead>
<tr>
<th>Group</th>
<th>1st day</th>
<th>21st day</th>
</tr>
</thead>
<tbody>
<tr>
<td>NG</td>
<td>23.28±1.25</td>
<td>33.51±1.32</td>
</tr>
<tr>
<td>MG</td>
<td>23.37±1.33</td>
<td>31.51±1.33*</td>
</tr>
<tr>
<td>JGG</td>
<td>22.99±1.52</td>
<td>33.10±1.20*</td>
</tr>
<tr>
<td>HDG</td>
<td>23.37±1.46</td>
<td>34.24±1.32*</td>
</tr>
<tr>
<td>LDG</td>
<td>23.34±1.37</td>
<td>33.29±1.41*</td>
</tr>
</tbody>
</table>

Notes: Compared with NG P<0.01; Compared with MG P<0.05; #P<0.01. Effects of Zibu Shenjing Fang on Serum GH and IGF-1 Contents in the Mouse
Compared with NG, serum GH and IGF-1 contents significantly decreased in the MG (P<0.05); Compared with the MG, serum GH and IGF-1 contents significantly increased in the Jingui Shenqi Wan group, the Zibu Shenjing high dose group and the low dose group (P<0.05 or P<0.01, Table 2).

Table 2. Effects of Zibu Shenjing Fang on GH and IGF-1 levels in serum of the mouse (x ± s, n=10)

<table>
<thead>
<tr>
<th>Group</th>
<th>GH (µg/L)</th>
<th>IGF-1 (µg/L)</th>
</tr>
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<tbody>
<tr>
<td>NG</td>
<td>10.25±0.89</td>
<td>108.38±11.99</td>
</tr>
<tr>
<td>MG</td>
<td>9.42±0.69*</td>
<td>97.30±6.57*</td>
</tr>
<tr>
<td>JGG</td>
<td>10.24±0.81*</td>
<td>117.53±12.32*</td>
</tr>
<tr>
<td>HDG</td>
<td>11.04±0.75*</td>
<td>126.37±10.70*</td>
</tr>
<tr>
<td>LDG</td>
<td>10.84±0.63*</td>
<td>118.44±14.47*</td>
</tr>
</tbody>
</table>

Notes: Compared with NG P<0.05, *P<0.01; Compared with MG P<0.05; #P<0.01.

Statistical Analysis
All the data were analyzed with SPSS17.0 statistical software and the results were expressed as x ± s, and one-way ANOVA was used for significant test, LSD test was used for comparison between groups, P<0.05 was regarded as significant difference.

RESULTS

General State of the Mouse
Withered hair, sparse hair, listless, tired and sleepy, slow reaction, arching the back, slow action, and reduction of ingestation and drinking amount, easily to be frightened, often crowding together, and so on gradually appeared in the mice of the MG and in the mice of other four groups, slower hair, rapid reaction and activity appeared and amount of ingestion and drinking significantly increased.

DISCUSSION

Essence is substance basis of all life activities of the human body and stored in the kidney. Performance and maintenance of physiological functions of various zang- and fu-organs invariably depend on the “essence” in the
Kidney. Over fearing would influence physiological activities of human zang- and fu-organs. Injury of essence-qi in the kidney finally leads to slow development, senilism and other kidney-essence insufficient syndrome. In the present study, according to the TCM theory about cause of disease and pathogenesis, “the great fright impairs the kidney energy”, and literature report on modeling of kidney deficiency syndrome,1 the method of “the cat frightening the mouse” combined with consuming swimming was used to injure the kidney-essence to establish the mouse kidney insufficiency model. In the experiment, withered hair and sparse hair, listless, tired and sleepy, slow reaction, arching the back, slow action, and reduction of ingestion and drinking amount, easily to be frightened, often crowding together, and so on appeared in the mice of the MG, which confirm to pathological state of kidney-essence insufficiency syndrome. Therefore, the model established in the study reflects dysfunction of the kidney dominating the essence, thus it can be used as the kidney essence deficiency syndrome model.

Kidney-essence stores in the kidney which is in charge of growth and development, and it is related with human birth, growth, development, senilism and death. Thus, in the present experiment, the body weight, an index of growth and development, was monitored. It was found that compared with the NG, the body weight of the mouse in the MG was reduced, and after the Zibu Shenjing Fang for tonifying the kidney and supplementing essence was administrated, the body weight of the mouse significantly increased, indicating that sufficient kidney-essence promotes growth and development, and insufficient kidney-essence slows down growth and development of the mouse. In addition, the endocrine system is of an important functions in maintaining homeostasis and regulating growth and development of the organism.2 In order to study the mechanism of tonifying the kidney and supplementing essence in promoting growth and development of the organism, serum GH and IGF-1 contents were detected. GH is released from the adenohypophysis and is regulated by growth hormone releasing hormone (GHRH) and growth hormone releasing inhibiting factor (GHIF) secreted by thalamus, and it is of functions of stimulating development of the organism, increasing volume and number of body cells, etc., and influence all tissue types of the organism, including bones, cartilages, adipose tissue, immune system and reproduction system, so, various organs develop and grow and perform functions under influence of GH, and the bones lengthen and the body gets higher.3-5 While biological effects of GH are mediated mainly by IGF-1 produced by the liver and other tissues, and IGF-1 as an important mitogen promotes growth and differentiation of cells. Therefore, GH and IGF-1 jointly constitute GH/IGF-1 promoting growth axis, which plays an important regulatory role in human growth and development.5 This is similar to the theory of TCM, essence stores in the kidney and the kidney is in charge of growth and development. In the present experiment, it was showed that in the model mouse, MG decreased, serum GH and IGF-1 contents significantly decreased, and in all the medicinal groups, the body weight increased and GH and IGF-1 contents significantly increased and the syndrome manifestations of the kidney-essence insufficiency mouse were significantly improved. It is suggested that kidney-essence insufficiency induces dysfunction of the GH/IGF-1 promoting growth axis system, and it is demonstrated that the Chinese drugs for tonifying the kidney and supplementing essence give play to the function of tonifying the kidney and supplementing essence through regulating the GH/IGF-1 promoting growth axis, and promote growth and development of the organism. However, the GH/IGF-1 promoting growth axis is a complicated system, and it is involved in negative feedback control among GHRH, GH, IGF-1, and control of cytokines on growth axis, interrelation between the growth and the sex gland axis and so on. Therefore, the nature of essence stores in the kidney and kidney-essence insufficiency syndrome, and the mechanism of tonifying the kidney and supplementing essence remain to be further studied.

REFERENCES


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