

Modernization of Traditional Medicine

Rat model for study of the essence of spleen deficiency and dampness in Chinese medicine

Xiao-Chun Han¹, Xu-Ming Ji¹, Lin Liu², Shuang Zhang¹, Li Sun¹, Qiu-Jian Feng¹, Shi-Jun Wang^{1*}

¹College of Traditional Chinese Medicine, Shandong University of Traditional Chinese Medicine, Jinan, China.

²Department of Special Examination, Affiliated Hospital of Shandong University of Traditional Chinese Medicine, Jinan, China.

*Correspondence to: Shi-Jun Wang, College of Traditional Chinese Medicine, Shandong University of Traditional Chinese Medicine, Jinan, China. E-mail: pathology@163.com.

Highlights

Spleen deficiency is often accompanied by dampness. Cytokines, immunoglobulins, and gastrointestinal hormones play major roles in the pathogenesis of spleen deficiency and dampness.

Editor's Summary

The two TCM syndromes “spleen deficiency” and “dampness” were cause and effect to each other.

Immunoglobulins

(IgA, IgG, C3)



Gastrointestinal Hormones

(GAS, MTL, VIP)



Water Metabolism

(ALD, ADH, ANP)



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Abstract

Objective: To explore the essence of spleen deficiency and dampness in Chinese medicine by successfully constructing a rat model with this syndrome. **Methods:** Rat models with the syndrome of dampness and spleen deficiency were established with the use of a high-fat, low-protein diet and excessive fatigue induced by loaded swimming. A total of 25 common clinical serological markers were tested. The T test, rank test, and partial least squares regression-discriminant analysis were used to analyze the data. **Results:** Total protein, albumin, motilin, interferon- γ , interleukin-2, immunoglobulin A, immunoglobulin G, and complement 3 levels in the model rats were lower than those in the control group ($P = 0.029$, $P = 0.032$, $P < 0.001$, $P < 0.001$, $P < 0.001$, $P < 0.001$, $P < 0.001$, respectively). The serum creatinine, total cholesterol, aldosterone, antidiuretic hormone, gastrin, interleukin-4, somatostatin, atrial natriuretic peptide, and vasoactive intestinal peptide levels in the model group were higher than those in the control group ($P < 0.001$, $P = 0.015$, $P < 0.001$, $P < 0.001$, $P < 0.001$, $P < 0.001$, $P < 0.001$, respectively). There was no statistical difference between the two groups in aspartate transaminase, alanine aminotransferase, globulin, albumin/globulin, blood urea nitrogen, high-density lipoprotein, and low-density lipoprotein. The model animal syndrome used two important variables in analysis with partial least squares regression-discriminant analysis. At the same time, the two dimensions were difficult to distinguish for each biological index. **Conclusion:** Spleen deficiency is often associated with dampness. The index that represents spleen deficiency can also represent dampness. Cytokines, immunoglobulins, and gastrointestinal hormones play a major causative role in both spleen deficiency and dampness. **Keywords:** Spleen deficiency, Dampness, Animal model

摘要

目的: 通过构建脾虚水湿证型的动物模型探讨中医“脾虚”和“水湿”的本质。

方法: 以高脂低蛋白因素和游泳过度疲劳因素诱导脾虚水湿动物模型, 选择临床常见的 25 项血清学指标进行检测, 采用 t 检验、秩检验和回归分析对数据进行分析。

结果: 模型组大鼠的总蛋白、白蛋白、胃动素、干扰素- γ 、白细胞介素-2、免疫球蛋白 A、免疫球蛋白 G、补体 3 水平均低于对照组 ($P = 0.029$, $P = 0.032$, $P < 0.001$, $P < 0.001$, $P < 0.001$, $P < 0.001$, $P < 0.001$, $P < 0.001$)。模型组的血清肌酐、总胆固醇、醛固酮、抗利尿激素、胃泌素、生长抑素、IL-4、心房钠尿肽和血管活性肠肽水平均高于对照组 ($P < 0.001$, $P = 0.015$, $P < 0.001$, $P < 0.001$, $P < 0.001$, $P < 0.001$, $P < 0.001$, $P < 0.001$)。两组在天冬氨酸转氨酶, 丙氨酸转氨酶、球蛋白、白蛋白/球蛋白、血尿素氮、高密度脂蛋白, 低密度脂蛋白水平无统计学差异。回归分析结果显示, 模型组动物证候具有二维性, 且此二维性在各生物学指标上难以明显分开。

结论: 脾虚常伴随水湿, 细胞因子、免疫球蛋白、及胃肠激素是影响脾虚水湿的重要因素。

关键词: 脾虚; 水湿; 动物模型

Abbreviation: PLS-DA, Partial least squares regression-discriminant analysis; Ig, Immunoglobulin; TCM, Traditional Chinese medicine; ALT, Serum alanine aminotransferase; AST, Aspartate transaminase; TP, Total protein; AP, Albumin; GP, Globulin; BUN, Blood urea nitrogen; SCr, Serum creatinine; TG, Triglyceride; TC, Total cholesterol; HDL, High-density lipoprotein; ALD, Aldosterone; ADH, Antidiuretic hormone; GAS, Gastrin; MTL, Motilin; IFN- γ , Interferon- γ ; IL-2, Interleukin 2; IL-4, Interleukin 4; C3, Complement 3; SS, Somatostatin; ANP, Atrial natriuretic peptide; VIP, Vasoactive intestinal peptide.

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Background

In traditional Chinese medicine (TCM), the spleen is the hub of water metabolism [1-3]. The spleen regulates the balance of water metabolism and digestion, absorption, and transportation of cereal essence. *Suwen Jingmaibielun* said: “spleen Qi can scatter fine through attributing to the lungs, smoothing the transfer channel and arriving at the lower bladder.” When spleen deficiency affects the formation, distribution, and excretion of body fluid, patients develop dampness syndrome.

Spleen deficiency and dampness syndrome, known as dampness-obstructing spleen-stomach syndrome, is a disorder of water and fluid caused by dampness trapped in the spleen and stomach, as well as repression of Qi function [4-5]. Spleen deficiency and dampness syndrome is very common. Many chronic diseases such as obesity, ulcerative colitis, diabetes, and tumors may be associated with the spleen deficiency and dampness syndrome, which leads to disease recurrence and persistence [6].

Spleen deficiency and dampness syndrome adversely affects the quality of life, and is an obstacle to the treatment of the disease [7]. The study of spleen deficiency and dampness syndrome was originally based on clinical symptoms, as modern medical biomarkers were unknown [8-9]. To explore the essence of “spleen deficiency” and “dampness”, we established a rat model with this syndrome to study the differences in serological values.

Materials and methods

Experimental animal and model establishment

Twenty Wistar rats (10 males, 10 females; specific-pathogen-free; 4 weeks old, weighing 150 ± 10 g) were randomly divided into two groups, with 10 rats in each group. The Wistar rats were purchased from Beijing Vital River Laboratory Animal Technology Co. Ltd. (Beijing, China). The animal certification number was No.11400700067086.

Feeding conditions: individually ventilated cage with temperature $23 \pm 2^\circ\text{C}$, humidity 40-70%, unrestricted diet and water. Feed was purchased from Beijing Huafukang Bioscience Co. Inc. (Beijing, China). The control rats were given AIN-76A purified rodent diet (ingredients: corn starch 15%, sucrose 50%, cellulose 5%, casein 20%, corn oil 5%, mineral mix 3.5%, vitamin mix 1%, DL-methionine 0.3%, and choline bitartrate 0.2%). The model rats were given a high-fat, low-protein diet (ingredients: sucrose 47.5%, starch 15%, lard 15%, casein 7%, cellulose 5%, corn oil 5%, mineral mix 3.5%, vitamin mix 1%, cholesterol 0.5%, choline bitartrate 0.2%, and DL-methionine 0.3%) and subjected to exhaustive swimming with a tail load (10% of body weight) every afternoon for 6 weeks (approved by the National Patent Office, No. 201410072183.8). Exhaustive swimming meant the nose was in water for

10s [2].

Experimental equipment and reagents

The following were used in the study: RT-2100C multi-functional automatic microplate reader (Shenzhen Lei Du company, Shenzhen China); YLS-1B multi-functional rat independent activity recorder (Shandong Academy of Medical Sciences, Shandong, China); UV-2201 trace ultraviolet spectrophotometer (Japan); Olympus AU5400 Automatic biochemical analyzer (Japan). Small Animal Anesthesia Machine (Matrx VIP3000, USA). Rat gastrin enzyme-linked immunosorbent assay kit (Beijing equation biology, Number 20130701).

Detection index

After 6 weeks, the model was established. The animals were weighed and sacrificed under isoflurane anesthesia. Serum alanine aminotransferase (ALT), aspartate transaminase (AST), total protein (TP), albumin (AP), globulin (GP), AP/GP, blood urea nitrogen (BUN), serum creatinine (SCr), triglyceride (TG), total cholesterol (TC), and high-density lipoprotein (HDL) levels were measured using an automatic biochemical analyzer. Aldosterone (ALD), antidiuretic hormone (ADH), gastrin (GAS), motilin (MTL), interferon- γ (IFN- γ), interleukin-2 (IL-2), interleukin 4 (IL-4), immunoglobulin (Ig) A, IgG, complement 3 (C3), somatostatin (SS), atrial natriuretic peptide (ANP), and vasoactive intestinal peptide (VIP) levels were measured using enzyme-linked immunosorbent assays.

The experiment was approved by the experimental animal center of Shandong University of Traditional Chinese Medicine; the care of laboratory animals and experimental surgery conformed to the Beijing Administration Rules for Laboratory Animal Care, 2004.

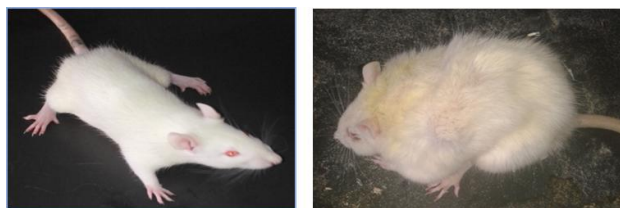
Statistical methods

All data in this study were checked in double-entry mode. SPSS 21.0 was used for statistical analysis. Animal weight, biochemical markers, and serological tests were recorded using the SIMCA11.5 software package. The model and control groups were assessed using partial least squares regression-discriminant analysis (PLS-DA). The T test and rank test were used to compare body weight and serology indices between the two groups and the data were described as mean $\pm$ standard deviation (SD). $P < 0.05$ was considered statistically significant.

Results

The appearance of model and control groups

Compared with the control group, the model rats had dull coats and were apathetic, with lower athletic ability, slow gait, weight loss, and thin, loose stools. Some rats had subcutaneous edema, with higher skin moisture content (Figure 1). The body weights of rats in the model group were higher than those in the control group ($P = 0.001$) (Table 1). The model rats reflected the state of dampness in Chinese medicine.



Control rat

Model rat

Figure 1 The appearance comparison of model group and control group

Comparisons of biological parameters

The TP, AP, MTL, IFN- γ , IL-2, IgA, IgG, and C3 levels in the model group were lower than those in the control group ($P = 0.029$, $P = 0.032$, $P < 0.001$, $P < 0.001$, $P < 0.001$, $P < 0.001$, $P < 0.001$, $P < 0.001$) (Table 1). The SCr, TC, ALD, ADH, GAS, IL-4, SS, ANP, and VIP levels in the model group were higher than those in the control group ($P < 0.001$, $P = 0.015$, $P < 0.001$, $P < 0.001$, $P < 0.001$, $P < 0.001$, $P < 0.001$, $P < 0.001$) (Table 1). AST, ALT, AST/ALT, GP, AG, BUN, HDL, and LDL levels were comparable between the two groups.

Table 1 The comparison of biological parameters between the model group and the control group

Biological parameters	Control group	Model group	t	P
Weight	340.90 $\pm$ 56.63	257.35 $\pm$ 44.65	3.833	0.001
ALT	40.88 $\pm$ 20.52	46.29 $\pm$ 18.24	-0.640	0.529
AST	129.50 $\pm$ 37.97	118.14 $\pm$ 47.60	0.576	0.571
AST/ALT	3.43 $\pm$ 0.97	2.63 $\pm$ 1.20	1.602	0.125
TP	59.50 $\pm$ 3.62	54.36 $\pm$ 5.49	2.356	0.029
AP	29.91 $\pm$ 1.66	26.79 $\pm$ 3.59	2.303	0.032
GP	29.59 $\pm$ 3.42	28.21 $\pm$ 3.39	0.909	0.378
AP/GP	1.03 $\pm$ 0.16	0.94 $\pm$ 0.10	1.490	0.152
BUN	5.25 $\pm$ 1.23	4.53 $\pm$ 1.94	0.945	0.356
SCr	20.50 $\pm$ 2.45	27.50 $\pm$ 5.32	-4.206	< 0.001
TG	0.38 $\pm$ 0.11	0.56 $\pm$ 0.26	-2.328	0.031
TC	1.94 $\pm$ 0.27	2.41 $\pm$ 0.45	-2.656	0.015
HDL	0.66 $\pm$ 0.09	0.67 $\pm$ 0.17	-0.019	0.985
LDL	0.27 $\pm$ 0.08	0.41 $\pm$ 0.25	-1.466	0.158
ALD	188.18 $\pm$ 19.80	371.22 $\pm$ 31.69	-14.696	< 0.001
ADH	4.10 $\pm$ 0.55	8.41 $\pm$ 0.63	-16.092	< 0.001
GAS	96.23 $\pm$ 12.39	196.30 $\pm$ 15.52	-15.569	< 0.001
MTL	221.77 $\pm$ 16.30	128.14 $\pm$ 19.63	11.400	< 0.001
IFN- γ	69.83 $\pm$ 5.49	35.57 $\pm$ 6.79	12.146	< 0.001
IL-2	914.69 $\pm$ 75.90	397.45 $\pm$ 86.15	14.111	< 0.001
IL-4	38.35 $\pm$ 4.91	79.70 $\pm$ 5.91	-16.725	< 0.001
IgA	218.38 $\pm$ 22.66	107.46 $\pm$ 18.55	12.461	< 0.001
IgG	8.89 $\pm$ 0.98	4.38 $\pm$ 0.69	12.745	< 0.001
C3	532.65 $\pm$ 57.02	212.88 $\pm$ 47.62	14.118	< 0.001
SS	35.36 $\pm$ 3.98	77.38 $\pm$ 7.84	-14.065	< 0.001
ANP	0.18 $\pm$ 0.02	0.29 $\pm$ 0.02	-15.342	< 0.001
VIP	93.57 $\pm$ 8.90	164.93 $\pm$ 11.76	-15.517	< 0.001

PLS-DA analysis

Body weight, serum protein, gastrointestinal hormones, cytokines, immunoglobulins and other indices were significantly different between the control rats and the model rats. All indices were imported into SIMCA 11.5 software and supervised pattern recognition analysis using PLS-DA was performed. The data showed two main variables of importance (VIP1, VIP2) (Figure 2). The first principal component was the main function of

performance, accounting for 96.3% of the syndromes (Table 2), the second principal component comprised 2.82% of the syndromes. The VIP1 and VIP2 may represent the two polarities (spleen deficiency and dampness). The index of the VIP1 can represent that of VIP2 since the two are interwoven. ALD, ADH, GAS, MTL, IFN- γ , IL-2, IL-4, IgA, IgG, C3, SS, ANP, VIP, and TP have a major effect on VIP1 and VIP2.

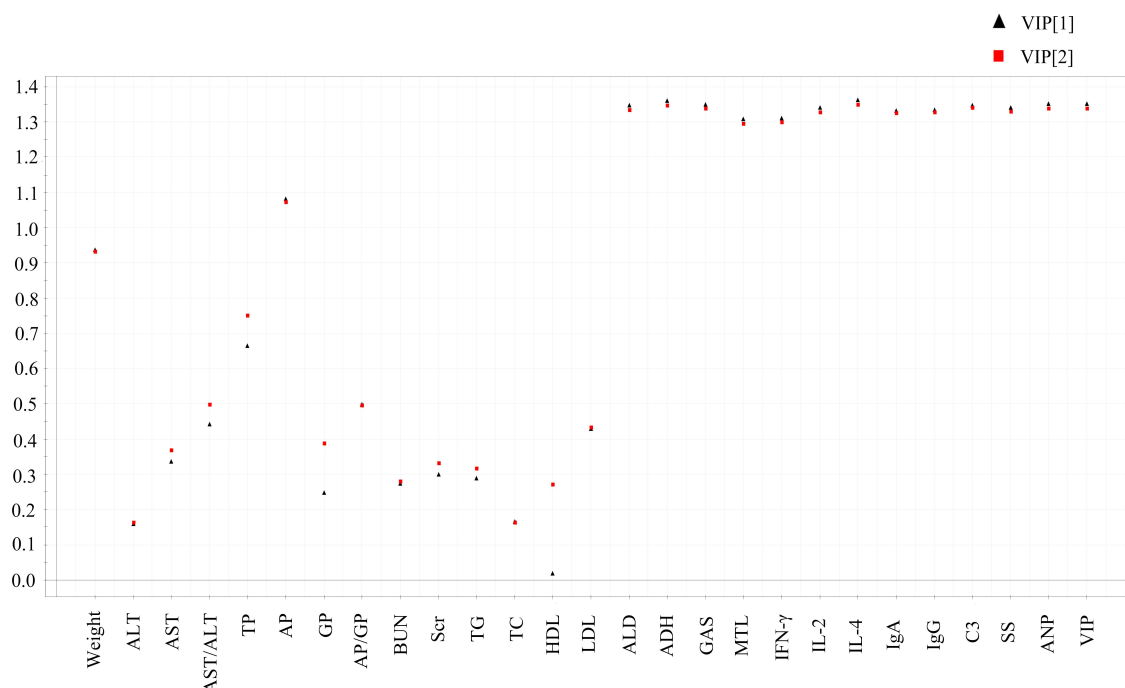


Figure 2 The VIP of PLS-DA analysis between the model group and the control group

The VIP1 and the VIP2 stand for the two polarities, combined with the theory of TCM, which may be “spleen deficiency” and “dampness”.

Table 2 PLS-DA analysis of the related indicators between the model group and the control group

Principal component	Eigenvalues	% of R ² X	Cumulative R ² X	% of R ² Y	Cumulative R ² Y
1	12.10	52.40	52.40	96.30	96.30
2	2.26	9.84	62.24	1.82	98.12

1 points to VIP1, 2 points to VIP2, R² means the contribution rate.

Discussion

According to the TCM theory, fatty food, excessive fatigue, excessive thinking, and prolonged innate weakness of the body are main causes of spleen deficiency [10, 11]. It has been confirmed that spleen deficiency will result in stagnation of water and dampness [12, 13]. This is the pathogenesis of spleen deficiency and dampness syndrome. A good animal model is needed for successful research.

In this study, a rat model with spleen deficiency and dampness syndrome was successfully constructed by feeding rats a high-fat, low-protein diet and subjecting them to exhaustive swimming with a tail load (10% of body weight) every afternoon for 6 weeks. This modeling method has been tested in other studies [14-16]. The composite indicators of the rat model with spleen deficiency and dampness syndrome, including general status, gastrointestinal function, immune function, etc., are very similar to those in spleen deficiency and dampness syndrome in humans under the influence of internal and external dampness [17-19].

In this study, we analyzed serum biochemical and

cytokine levels that reflect the syndrome to determine the specificity and stability of clinical diagnosis for spleen deficiency and dampness. The results showed that TP and AP were lower while TG and TC were higher in the rat model, which agreed with the TCM theory. A high-fat diet and excessive fatigue led to “internal dampness damaging the spleen” [20]. Thus, blood lipid levels were higher while blood protein levels were lower. This is one manifestation of spleen deficiency and dampness.

Cytokines and gastrointestinal hormones showed significant differences. IL-2 and IFN- γ levels were lower and IL-4 levels were higher, indicating that the rats showed lymphocyte Th1/Th2 equilibrium drift. IgG, IgA, and C3 levels were lower. The abnormal humoral immune indices were in accordance with IL-2 and IL-4 levels. Jiang reported that patients with spleen deficiency had similar changes [21]. The rat model presented a syndrome similar to spleen deficiency. The cytokines and the humoral immune indices reflected “spleen deficiency” syndrome in the model animal [22].

ALD, ADH, and ANP can affect water absorption and influence urine volume. Changes in urine volume can reflect metabolism and accumulation of water [23]. The study showed that the level of ADH in the model group

was higher, and the volume of urine was lower, indicating that the body showed water accumulation, which reflected the “dampness” syndrome in the model animal.

PLS-DA analysis can summarize data using a supervised analysis method [24]. The result showed two polarities (VIP1, VIP2), indicating that the model rats have two distinct characteristics. According to the model, VIP1 and VIP2 represent “spleen deficiency” and “dampness”, respectively. VIP1 was the first principal component, accounting for 96.3% of the performance of the model animal syndrome (Table 2), representing “spleen deficiency”. VIP2 was the second principal component, accounting for 2.82% of the performance of the model animal syndrome, representing “dampness”.

Meanwhile, VIP1 and VIP2 showed commonality. Figure 2 showed that weight, AP, ALD, ADH, ANP, cytokines, and gastrointestinal hormones were significantly associated with VIP1 and VIP2, while ALT, AST, Scr, and BUN showed a smaller association. This result indicated that “spleen deficiency” and “dampness” syndromes appearing in the model animals had similar indicators. The indicators reflecting “spleen deficiency” can also reflect “dampness.” This further confirms the correctness of the dialectical method of the two viscera in the TCM theory of spleen deficiency induced by dampness and dampness induced by spleen deficiency, which is in good agreement with the results of a population-based study [25, 26]. “Spleen deficiency” and “dampness” were cause and effect to each other. This would explain the link between the two TCM syndromes shown by the biological indicators and data distribution. The TCM theory may be interpreted and analyzed using modern medical and statistical methods.

Conclusion

Combined with the model evaluation and TCM theory, and taking into account the mechanism of spleen deficiency and dampness, we believe that: (1) spleen deficiency is often accompanied by dampness; (2) the index representing spleen deficiency can also represent dampness; and (3) cytokines, immunoglobulins, and gastrointestinal hormones play major roles in the pathogenesis of spleen deficiency and dampness.

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