Correlation between pathogenesis of dampness syndrome and Interleukin-2, Interleukin-8 in rats

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Abstract

OBJECTIVE: To explore the pathogenesis of dampness syndrome by detecting the changes of interleukin-2 (IL-2) and interleukin-8 (IL-8) levels.

METHODS: Female Sprague Dawley rats were divided randomly into five groups according to the random number table: a normal group (Group I), an external dampness group (Group II), an internal dampness group (Group III), and an external and internal dampness group (Group IV). Twenty days after the model made, IL-2 and IL-8 levels were detected by radioimmunoassay method.

RESULTS: The IL-2 and IL-8 levels among groups were significant (F=3.102, P<0.05; F=2.657, P<0.05, respectively). The level of IL-2 in Group II and Group III were higher than that in Group I (P<0.05, P<0.01, respectively), especially higher in the Group III compared with Group II (P<0.05). The level of IL-8 in Group III were higher than those in Group I, Group II and Group IV (P<0.05, P<0.01, P<0.05, respectively). In the Group III, the 24-hour water and body weight were higher than that in the Group IV (all P<0.05), and spontaneous movement frequency was higher than those in Group II and Group IV (P<0.05).

CONCLUSION: Immune activation and inflammatory reaction might be easily caused by external dampness other than internal dampness.

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Key words: Interior dampness syndrome; Excessive dampness syndrome; Interleukin-2; Interleukin-8

INTRODUCTION

Environmental dampness has a wide effects on health.1-3 Dampness is distinguished into "external dampness" and "internal dampness" in Traditional Chinese Medicine (TCM), external dampness is induced in many cases by environmental dampness, while internal dampness refers to the imbalance of metabolism due to dysfunction of liquid and humor.4-6 Correlation between environmental dampness and several diseases have been identified in several epidemiological studies.1,2,7 The effect of dampness exposure on immune function is manifold and numerous. Several studies have shown that humidity stress is associated with suppression of several T-cell functions and defective immune response.8-10 However, whether the effect of external dampness and internal dampness on cellular immune function of rats is consistent or not remains obscure. The aim of the present study is to elucidate the possible effects of dampness on Interleukin-2 (IL-2), Interleukin-8 (IL-8) in rats.
MATERIALS AND METHODS

Animals
Female Sprague Dawley (SD) rats, weighing (230 ± 10) g, were obtained from Animal Experiment Center of Sun-yat sen University. All procedures were performed according to the Institutional Guidelines for the Care and Use of Laboratory Animals. The study protocol was approved by the Ethics Committee of The First Affiliated Hospital of Sun Yat-Sen University (Guangzhou, China). The rats were randomly housed in groups of four per wire-mesh cage (39 cm × 26 cm × 21 cm) for at least 1 week.

Experimental design
Forty-eight SD rats were randomly divided into five groups, with 8 rats in each group:

Group I (normal group): rats were placed in a controlled environment of (22°C±1°C) and (55%±5%) relative humidity with free access to standard food in pellets (supplied and designed by Animal Experiment Center of Sun-yat sen University) and tap water, on a 12 h light/dark cycle throughout 20 days.

Group II (internal dampness group): rats were placed in a controlled environment of (23°C±1°C) and (55%±5%) relative humidity with free access to high glucose and fat of food in pellets (supplied and designed by Animal Experiment Center of Sun-yat sen University) and 2 ml frozen water intragastrically once every day, on a 12 h light/dark cycle throughout 20 days.

Group III (external dampness group): rats were placed in a controlled environment of (26°C±1°C) and (94%±5%) relative humidity with free access to high glucose and fat of food in pellets (supplied and designed by Animal Experiment Center of Sun-yat sen University) and 2 ml frozen water intragastrically once every day, on a 12 h light/dark cycle throughout 20 days.

Group IV (both internal and external dampness group): rats were placed in a controlled environment of (26°C±1°C) and (94%±5%) relative humidity with free access to high glucose and fat of food in pellets (supplied and designed by Animal Experiment Center of Sun-yat sen University) and 2 ml frozen water, on a 12 h light/dark cycle. Let the rats swimming in 20 cm depth pool with temperature between 25°C-27°C for 15 min every day throughout 20 days.

In each animals, 24-hour food intake, 24-hour water intake, body weight as well as spontaneous movement frequency among the four groups at the beginning of the trial.

At the end of the trial, 24-hour food intake of the Group II and Group IV were lower than that in the Group I (both P<0.05). 24-hour water intake of the Group II, Group III and Group IV were lower than that in the Group I (all P<0.01), 24-hour water intake of the Group III was higher than that in the Group IV (P<0.05). Body weight of the Group II, Group III and Group IV were lower than that in the Group I (P<0.01, P<0.05, P<0.01, respectively), and the Group III was higher than the Group IV (P<0.05). Spontaneous movement frequency of the Group II, Group III and Group IV were lower than that in the Group I (all P<0.01), and the Group III was higher than the Group II and Group IV (all P<0.05) (Table 1).

Levels of IL-2 and IL-8 in all groups
The level of IL-2 in the Group II and Group III were higher than that in the Group I (P<0.05, P<0.01, respectively), and the Group III was higher than the Group II (P<0.05). The level of IL-8 in the Group III was higher than the Group I (P<0.05, P<0.01, respectively), and the Group III was higher than the Group II (P<0.05).

Cytokine measurements
Two milliliters of blood was collected by intracardiac puncture. Each sample contained 0.22 ml 6-keto EDTA, and was centrifuged for 15 min at 5500 r/min. The plasma from each sample in the tubes was stored at -80°C. Serum level of IL-2 and IL-8 were determined by Radioimmunoassay (RIA) with the IL-2 and IL-8 RIA kits (Beijing Furui Biological Engineering Company, Beijing 100085, China) according to their manufacturer’s instructions.

Statistical analysis
Software SPSS 13.0 was used in all statistical analyses. Data were expressed as mean±standard deviation. The analysis of variance (ANOVA) was used to determine significant differences among groups, and comparison between two groups by LSD test. P<0.05 was considered to be significant.

RESULTS
Changes of symptoms and physical sign
Except in Group 1, symptoms of dampness appeared in rats of all the model groups about 5-7 days during the model-making process, with the GroupIV the most obviously appeared.

There was no difference in the 24-hour food intake, 24-hour water intake, body weight, spontaneous movement frequency among the four groups at the beginning of the trial.

1) apathetic, lying hobbies, lazy acting (sticking together); 2) inappetence; 3) loose stool; 4) reduced drinking. The animal models showed symptoms analogous to humans, such as fatigue, loss of appetite, change of fur color and weight loss.
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was higher than that in the Group I, Group II and Group IV (P<0.05, P<0.01, P<0.05, respectively) (Table 2). Compared among all the groups, there were significant difference in the levels of IL-2 and IL-8 (F=3.102, P<0.05; F=2.657, P<0.05, respectively).

### DISCUSSION

Dampness is characterized by its impediment to Qi movement and its turbidity, heaviness, stickiness, and downward-flowing properties. The dampness of TCM includes "external dampness" and "internal dampness". External dampness is induced by environmental in many cases, typically during damp weather or when a person comes into contact with moisture for extended periods of time, as with high humidity, sweaty clothes or wet or damp environments. Occupations such as gardening, working in a laundry and dishwashing may be fertile grounds for diseases of dampness. Internal dampness is likely to occur by an overtake of excessive sweets, dairy, starchy and glutinous foods, greasy or fried foods, watery fruits and vegetables, and alcohol, raw, cold, greasy, or sweetened food. Internal dampness is usually accompanied by a weakness in the body’s digestive system. Responsible for separating food into nutrition and waste, a weak digestive system causes the excretion of nutritional elements, and allows unhealthy substances to remain in our systems. When a weak digestive system allows material that should be excreted to circulate, proper fluid movement is hampered, causing dampness. However, the symptoms associated with dampness are not too imme-

Table 1 Changes of 24-hour food intake, 24-hour water intake, body weight and spontaneous movement frequency in all groups (mean ± s)

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Food intake (g/kg)</th>
<th>Water intake (g/kg)</th>
<th>Body weight (g)</th>
<th>Movement (frequency)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>8</td>
<td>120±13</td>
<td>2080±405</td>
<td>280±13</td>
<td>886±50</td>
</tr>
<tr>
<td>II</td>
<td>8</td>
<td>100±11'</td>
<td>147±30'</td>
<td>238±11'</td>
<td>591±143'</td>
</tr>
<tr>
<td>III</td>
<td>8</td>
<td>108±13</td>
<td>160±33'</td>
<td>258±11'</td>
<td>6512±47'</td>
</tr>
<tr>
<td>IV</td>
<td>8</td>
<td>957±11'</td>
<td>133±30'</td>
<td>229±11'</td>
<td>5076±39'</td>
</tr>
</tbody>
</table>

Notes: Group I refers to normal group, Group II refers to internal dampness group, Group III refers to external dampness group, and Group IV refers to internal and external dampness group; food intake refers to 24-hour food intake, water intake refers to 24-hour water intake, and movement refers to spontaneous movement frequency. Compared with Group I, *P<0.05; **P<0.01; compared with Group III, *P<0.01, **P<0.05.

Table 2 Levels of IL-2 and IL-8 in all groups (mean ± s)

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>IL-2 (µg/L)</th>
<th>IL-8 (µg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>8</td>
<td>0.77±0.55'</td>
<td>0.50±0.19'</td>
</tr>
<tr>
<td>II</td>
<td>8</td>
<td>0.91±0.50'</td>
<td>0.37±0.23'</td>
</tr>
<tr>
<td>III</td>
<td>8</td>
<td>1.81±0.98'</td>
<td>0.83±0.53'</td>
</tr>
<tr>
<td>IV</td>
<td>8</td>
<td>1.57±0.79'</td>
<td>0.50±0.15'</td>
</tr>
</tbody>
</table>

Notes: Group I refers to normal group, Group II refers to internal dampness group, Group III refers to external dampness group, and Group IV refers to internal and external dampness group. Compared with Group III, *P<0.01, **P<0.05; compared with Group I, *P<0.01, **P<0.05.

Several epidemiological studies have identified the correlations between environmental dampness and several diseases, such as allergic diseases, skin diseases, stroke and respiratory symptoms. Also, lifestyle factors, especially unhealthy eating patterns, may affect human health. The effect of humidity exposure on immune function have been identified, and several studies have shown that dampness stress is associated with suppression of several T-cell functions and defective immune response. However, whether the effect of external dampness and internal dampness on cellular immune function of rats is consistent or not remains obscure. As we all know, IL-2 and IL-8 are important inflammatory and immune media. IL-2 is a protein that attracts lymphocytes and is necessary for the growth and function of T cells. IL-2 is normally produced by the body during an immune response and plays a pivotal role in for the expansion of most T-cells, natural killer cells and B cells during certain phases of their response. Therefore, it is part of the body’s natural response in discriminating between foreign (non-self) and self and plays a central role in antimicrobial infection and antitumor effects. IL-8 is a chemokine produced by lymphocytes, neutrophils, epithelial cells, vascular cells, and fibroblast, which involved in leucocyte recruitment and activation. It is increased in a broad range of acute inflammatory disorders and has chemotactic activity for T lymphocytes. Therefore, IL-8 might be link between neuropeptides and inflammatory mediators and is an important mediator of the immune reaction in the innate immune system response. However, most studies about IL-2 and IL-8 in modern medicine only focused on pathogens or diseases, while the study on the association between dampness and IL-2 and IL-8 is very few.

In previous studies, we found dampness syndrome was closely related with decreased T immune function in chronic hepatitis B patients. In the present study, we discovered that the level of IL-2 in external dampness group and internal dampness group were higher than those in normal group, which indicated that dampness was closely relative to immune activation and inflam-
matory reaction in the period of dampness syndrome. It was interesting to find that IL-2 and IL-8 levels of external dampness group were higher than those in internal dampness group, which indicated that the immune of body might be more sensitive to the stimulation of external dampness compared with that of internal dampness. It is difficult to explain why the level of IL-8 in external dampness group were higher than that in internal and external dampness group, because both internal dampness and external dampness could improve the level of IL-8 in our study. Whether external dampness is easy to be recognized by innate immune to produce effective immune response against related antigens, or internal and external dampness can not easily be recognized by innate immune to produce effective immune response against related antigens, are still unknown.

Similarly, we found 24-hour the amount of drinking water of external dampness group was higher than those in the internal and external dampness group, body weight of external dampness group was higher than that in internal and external dampness group, spontaneous movement frequency of external dampness group was higher than those in internal dampness group and internal and external dampness group. It might suggest that internal dampness might cause worse effect on health than external dampness. However, in our study, death didn’t appear in external dampness group. The reason for this was probably due to the shorter experimental time. It is worthy of further study on the changes of IL-2 and IL-8 under longer stimulated duration with external dampness. In conclusion, these findings suggested that immune activation and inflammatory reaction might be easily caused by external dampness other than internal dampness. Further studies would be needed to clarify the cause of these differences between internal dampness and external dampness.

REFERENCES
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