Effects of Immunostimulators on Involution of Lymphoid Organs in Mice Exposed to Heat and Cold Stress

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ABSTRACT. The effects of two immunostimulators, active egg white product (AEWP) and dihydroheptaprenol (DHP), on involution of lymphoid organs were investigated in mice exposed to heat and cold stress. Heat (37°C, 45 min) and cold (4°C, 4-24 hr for 4 days) stress induced involution of the thymus and/or spleen. AEWP was administered orally at a dose of 500 mg/kg for 4 consecutive days after exposure to heat stress or during cold stress. DHP was injected intramuscularly at a dose of 100 mg/kg on the 4th day after exposure to heat or cold stress. In mice exposed to heat and cold, the ratio of thymus to body weight was significantly higher in the AEWP- and DHP-treated groups as compared to the non-treated group (P<0.01), also the ratio of spleen to body weight was significantly higher in the DHP-treated group as compared to the non-treated group (P<0.01). These results showed that AEWP and DHP markedly alleviated involution of the thymus and/or spleen due to heat and cold stress. — KEY WORDS: cold and heat stress, immunostimulator, lymphoid organ.


It is generally accepted that environmental stressors are involved in the pathogenesis of infectious diseases in domestic animals [13]. Numerous animal studies have indicated that the immune system is affected by responses to stress [10]. A number of investigations have documented stress-induced physiological, hormonal, and immunological changes. For example, cortisol injection [18], heat stress [7] and cold stress [3-6] can cause involution of lymphoid organs such as the thymus, spleen, and lymph nodes through activation of the pituitary-adrenocortical axis [20].

Therefore, enhancement of the immune response may be a desirable therapeutic approach to the prevention or treatment of various infections in animals with apparent stress-induced immunosuppression. Many biological response modifiers (e.g., physiological products, microbial substances and synthetic compounds) have been tested for use as immunopotentiators and immunomodulators in domestic animals [15, 17].

Recently, it has been suggested that active egg white product (AEWP) [2] and dihydroheptaprenol (DHP) [1] possess immunostimulating activity. For example, these immunostimulators were shown to enhance non-specific host defense mechanisms against a variety of bacterial infections in healthy and immunosuppressed mice by stimulating the generation of neutrophils and also enhancing neutrophil function [1-2, 14, 16].

In the present study, the protective effects of AEWP and DHP with respect to stress-induced immunosuppression and involution of lymphoid organs were investigated in mice exposed to heat and cold stress.

Inbred male mice (CRI: BALB/c strain) were obtained from Charles River Breeding Laboratory, Inc. (Atsugi, Japan) and used at 8 weeks of age when weighing 17 to 22 g. The mice were maintained at 22±2°C with a 12 h light-dark cycle, and were supplied with standard laboratory feed (Oriental Yeast Co., Tokyo, Japan) and water ad libitum.

Groups of 20 mice were placed in an incubator maintained at 37°C for 45 min in order to expose heat stress.

Groups of 20 mice were placed in a low temperature room maintained at 4°C for 4 consecutive days in order to expose cold stress. Briefly, the mice were exposed to cold stress for 4, 7, 24 and 24 hr on the 1st day, 2nd day, 3rd day and 4th day, respectively.

AEWP was prepared as previously described [2, 14, 16]. In brief, chicken egg white was fermented with Saccharomyces cerevisiae and spray dried. Each lot of dried egg white powder was tested for protective effects against Escherichia coli infection in mice and only active lots with a significant protective effect (P<0.05 by the chi-square test) were used. AEWP (Neurich®) was supplied by Eisai Co., Ltd., Tokyo, Japan. Groups of 20 mice received AEWP orally at a dose of 500 mg/kg (emulsified in distilled water) once daily for 4 days after exposure to heat stress or during cold stress.

DHP was chemically synthesized and prepared as a microemulsion with lecithin at a 1% concentration by the Research Laboratories, Eisai Co., Ltd., Tokyo, Japan [1]. The chemical structure of DHP is shown in Fig. 1. Mice were intramuscularly injected with DHP at a dose of 100 mg/kg on the 4th day after exposure to heat or cold stress. Mice given the same volume of vehicle alone was used as controls.

Organ weight of mice were individually measured 1 day after the last dose of immunostimulator. The mice were sacrificed by ether anesthesia and the thymus and spleen removed immediately and weighed.

All data in the table represent mean value ± standard deviation for the indicated number of mice. The significance of differences between means was determined by Student's test.

CH₃
H⁺CH₂-C≡CH-CH₂-CH₂OH

CH₃

C₃₅H₆₀O

Fig. 1. Chemical structure of dihydroheptaprenol (DHP).

M.W.~496.86.
Table 1. Effects of immunostimulators AEWP and DHP on involution of lymphoid organs of mice exposed to heat and cold stress

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of mice</th>
<th>Body weight (g)</th>
<th>Thymus weight (mg)</th>
<th>Spleen weight (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>20</td>
<td>21.4 ± 1.4</td>
<td>58.8 ± 7.9**</td>
<td>89.6 ± 9.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(0.28 ± 0.04)**</td>
<td>(0.42 ± 0.03)**</td>
</tr>
<tr>
<td>Heat</td>
<td>20</td>
<td>20.9 ± 1.8</td>
<td>43.9 ± 5.4</td>
<td>88.7 ± 7.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(0.21 ± 0.03)</td>
<td>(0.42 ± 0.04)</td>
</tr>
<tr>
<td>Heat + AEWP(a)</td>
<td>20</td>
<td>20.8 ± 1.8</td>
<td>70.1 ± 12.0**</td>
<td>91.4 ± 10.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(0.34 ± 0.05**</td>
<td>(0.44 ± 0.04)</td>
</tr>
<tr>
<td>Heat + DHP(c)</td>
<td>20</td>
<td>21.2 ± 1.5</td>
<td>65.3 ± 6.6**</td>
<td>98.9 ± 8.2**</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(0.31 ± 0.04**)</td>
<td>(0.47 ± 0.03**)</td>
</tr>
<tr>
<td>Control</td>
<td>20</td>
<td>20.0 ± 1.1</td>
<td>66.2 ± 7.2**</td>
<td>91.0 ± 9.1**</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(0.33 ± 0.03**)</td>
<td>(0.46 ± 0.05**</td>
</tr>
<tr>
<td>Cold</td>
<td>20</td>
<td>19.7 ± 1.1</td>
<td>41.5 ± 6.5</td>
<td>82.3 ± 6.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(0.21 ± 0.03)</td>
<td>(0.42 ± 0.05)</td>
</tr>
<tr>
<td>Cold + AEWP(b)</td>
<td>20</td>
<td>19.7 ± 0.9</td>
<td>64.0 ± 10.3**</td>
<td>87.1 ± 7.7*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(0.33 ± 0.05**)</td>
<td>(0.44 ± 0.04)</td>
</tr>
<tr>
<td>Cold + DHP(c)</td>
<td>20</td>
<td>19.5 ± 1.5</td>
<td>59.8 ± 7.1**</td>
<td>91.0 ± 9.1**</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(0.31 ± 0.03**)</td>
<td>(0.47 ± 0.04**</td>
</tr>
</tbody>
</table>

Data are represented as mean ± standard deviation.

a) Ratio of lymphoid organ to body weight (%).
b) Active egg white product.
c) Dihydroheptaprenol.
*P<0.05; **P<0.01 as compared to heat or cold stress group.

Table 1 shows the thymus, spleen, and body weights of mice subjected to the various experimental conditions and treated with AEWP and DHP. There were no significant differences in body weight between heat or cold stress group and the other groups. Mice exposed to heat stress displayed significantly decreased thymus weight (P<0.01), but no significant decrease in spleen weight. AEWP and DHP treatment prevented reduction of thymus weight (P<0.01), which was maintained at more than the control level, and DHP-treated mice displayed an increase in spleen weight compared to heat stress mice (P<0.01). When the thymus weight was expressed as a percentage of body weight, a significant difference was found between the immunostimulator-treated and non-treated groups (P<0.01).

The weights of both thymus and spleen were reduced in cold stress-exposed mice (P<0.01). AEWP and DHP treatment prevented reduction of lymphoid weight, which was maintained at a nearly normal level. Furthermore, the weights of the thymus and spleen were significantly higher in all the immunostimulator-treated groups as compared to cold stress group (P<0.05 or P<0.01). However, no difference was found between the AEWP-treated and non-treated groups when the spleen weight was expressed as a percentage of body weight.

Stress is known to induce involution of lymphoid organs such as the thymus, spleen, and lymphoid nodes [18]. This involution serves as a common index for assessing the immunosuppressive effects of stress. The purpose of these experiments was to determine whether the immunostimulators AEWP and DHP can prevent this stress-induced involution.

In both stress paradigms, AEWP and DHP treatment were found to be effective in preventing the involution of lymphoid organs. Thus, the results indicate that AEWP and DHP are effective in protecting mice from stress-induced immunosuppression.

In heat and cold stressed animals, primary lymphoid organs such as the thymus and spleen undergo involution, which forms a part of the general adaptation syndrome. It has been suggested that the lymphocytes are redistributed by circulation to the peripheral lymphoid organs under the influence of corticosteroids [11, 12].

On the other hand, in vivo administration of steroid hormones decreases the number of circulating T and B lymphocytes [19]. Also, blastogenesis induced by a variety of mitogens is readily suppressed by pharmacologic doses of steroid hormones both in vivo and in vitro [8]. Furthermore, steroid hormones are reported to decrease neutrophil and macrophage functions such as chemotaxis and intracellular killing [9, 17].

A variety of stress paradigms have been shown to exacerbate the effects of several infectious agents [13]. AEWP and DHP were shown to enhance non-specific host defence mechanisms against Escherichia coli infection in mice treated with immunosuppressants such as cyclophosphamide and cortisone by stimulating the generation of neutrophils and also enhancing neutrophil and macrophage functions [1, 14, 16]. These studies demonstrated that AEWP and DHP could prevent death.
following systemic infection with *E. coli* in an immunosuppressed state.

In conclusion, the present study provides direct evidence of the protective effects of AEW and DHP as anti-stress agents. Further investigations of the effect of AEW and DHP on inhibiting the production and activity of glucocorticoids are in progress in our laboratories.

REFERENCES