IMMUNOSUPPRESSIVE ACTIVITIES OF 15-DEOXYSPERGUALIN IN ANIMALS

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Spergualin, which is a metabolite of Bacillus laterosporus1, has immunosuppressive activities2-5 as well as antitumor activity. The study of analogues revealed that 15-deoxyspergualin is one of the most active compounds. We report here various immunosuppressive activities of 15-deoxyspergualin in rodents.

Female CDF\textsubscript{i} mice were purchased from Shizuoka Laboratory Animal Center, Shizuoka, Japan. Male Fisher 344 and SHR rats were from Charles River Japan, Kanagawa, Japan. Spergualin and 15-deoxyspergualin were prepared at Takara Shuzo Co., Ltd.\textsuperscript{4,5}. They were dissolved in saline, and the solutions were sterilized by passing through a 0.22-\mu m filter membrane and stored at -20°C before use.

For the measurement of antibody production to sheep red blood cells (SRBC, Japan Biosupp Center, Tokyo, Japan), plaque-forming cells (PFC) were directly enumerated according to Cunningham and Szenberg\textsuperscript{6}. Tests for delayed-type hypersensitivity (DTH) to SRBC in mice and rat skin transplantation were carried out by the method of Ishizuka et al\textsuperscript{7} and a method described in the previous report\textsuperscript{8}, respectively. Data were statistically analyzed by

### Table 1. The effect of 15-deoxyspergualin on the production of antibody and DTH to SRBC in mice.

<table>
<thead>
<tr>
<th>15-Deoxyspergualin (mg/kg)</th>
<th>PFC (number/1 \times 10^6 spleen cells)</th>
<th>Footpad thickness increase (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1,122±156</td>
<td>1.51±0.28</td>
</tr>
<tr>
<td>1</td>
<td>561±141*</td>
<td>1.61±0.34</td>
</tr>
<tr>
<td>3</td>
<td>171±59*</td>
<td>0.79±0.35*</td>
</tr>
<tr>
<td>26±18*</td>
<td></td>
<td>0.11±0.21*</td>
</tr>
</tbody>
</table>

Female CDF\textsubscript{i} mice of 6 weeks old were used in each experiment. A group consisted of 5 to 7 mice. For the production of antibody, SRBC (1 \times 10^8) were intravenously (iv) injected on day 0. 15-Deoxyspergualin was intraperitoneally (ip) administered once a day for 3 days starting one day after the immunization. On day 4 the spleen cells were removed from the mice and subjected to the assay of PFC producing anti-SRBC. For the induction of DTH, SRBC (1 \times 10^5) was iv injected on day 0. 15-Deoxyspergualin was ip administered on the same schedule. On day 4 the mice were challenged by subcutaneous injection of 1 \times 10^8 SRBC into a footpad. Twenty four hours later the thickness of the footpad was measured with calipers. All data are shown as mean±SD.

* P<0.01.

### Table 2. The effect of the administration period of 15-deoxyspergualin on the mean survival time (MST) of skin allograft in recipient rats.

<table>
<thead>
<tr>
<th>Administration period (days)</th>
<th>Number of recipient rats</th>
<th>MST with SD (days)</th>
<th>MST with SD after completion of administration (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (Control)</td>
<td>5</td>
<td>7.2±0.8</td>
<td>—</td>
</tr>
<tr>
<td>10</td>
<td>8</td>
<td>16.0±3.0*</td>
<td>6.1±2.7**</td>
</tr>
<tr>
<td>20</td>
<td>6</td>
<td>29.7±3.7*</td>
<td>9.7±3.7**</td>
</tr>
<tr>
<td>30</td>
<td>5</td>
<td>45.6±0.5*</td>
<td>15.6±0.5***</td>
</tr>
</tbody>
</table>

Skin transplantation was carried out as described in the legend of Fig. 1. 15-Deoxyspergualin was ip administered at 6.25 mg/kg once a day starting one day after the transplantation.

* P<0.01 vs. control.
** P<0.05 vs. the value for rats administered for 10 days.
*** P<0.01 vs. the value for rats administered for 20 days.
As shown in Table 1, 15-deoxyspergualin significantly inhibited the production of antibody to SRBC at 1 to 10 mg/kg, when administered daily for 3 days starting one day after immunization with SRBC. It also inhibited DTH to SRBC at 3 and 10 mg/kg using the same administration schedule. In both cases inhibition was more than 92% at the highest dose.

The effects of spergualin and 15-deoxyspergualin on survival of SHR skin allograft in Fisher 344 recipients are compared in Fig. 1. 15-Deoxyspergualin significantly prolonged the mean survival time (MST) of skin grafts at lower doses than spergualin when administered daily for 10 days starting one day after the transplantation. The MST of skin grafts was further examined for rats given 6.25 mg/kg of 15-deoxyspergualin daily for 10, 20 and 30 days after grafting (Table 2). The MSTs were prolonged up to 16.0, 29.7 and 45.6 days, respectively. Thus, it was found that 15-deoxyspergualin markedly suppressed graft rejection, but stopping drug administration caused rejection of the grafts. However, the period between the termination of administration and the graft rejection was prolonged proportionally with the length of the administration period.

Recently, we reported that spergualin suppressed various immune responses2). In the present study 15-deoxyspergualin was demonstrated to inhibit both humoral and cell-mediated immune responses, and in the rat skin allotransplantation, 15-deoxyspergualin showed stronger activity than spergualin to inhibit the skin graft rejection.

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References