SUBACUTE TOXICITY OF (-)15-DEOXYSPERGUALIN IN BALB/c MICE. II. HISTOPATHOLOGICAL STUDY

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Abstract: Following the preceding report on hematological aspects in subacute toxicity of (-)15-deoxypergualin (DSP), the present report dealt with the histopathological changes produced by DSP, 0.5-5.0 mg/kg, given to BALB/c mice for three months. At sacrifice, various internal organs such as heart, lungs, liver, spleen and kidneys, were taken, and their wet weights immediately measured. HE- or PAS-stained sections were histopathologically studied under light microscope. Additionally, frozen sections of the spleen were prepared to evaluate the effect of DSP on the lymphocyte surface markers like thy 1 and B220 by avidin-biotin complex (ABC) technique. Although the mean weights of lungs, liver, spleen and kidneys in the 0.5 mg- and 2.5 mg-DSP groups were not significantly different from those in the control animals, the weights of the heart, lungs, liver and spleen in the 5 mg-DSP group were significantly lower. Histopathological studies by light microscopy revealed no abnormalities in the heart, lungs, liver or kidneys taken from the mice given 0.5-5.0 mg/kg DSP. In contrast, significant changes were observed in the spleen and bone marrow of the 5.0 mg group of mice. Likewise, in the intestine of the 0.5-5.0 mg groups dose-dependent lesions, such as degeneration or disappearance of the mucosal epithelium, infiltration by inflammatory cells, and pseudo-membrane formation, was observed. By ABC technique, preferential decrease of B cells was seen in the splenic corpuscles of the DSP-treated mice. Histopathological changes due to DSP predominantly seen in the lymphoid and/or hematopoietic organs may be directly related to the immunosuppressive potency inherent to this drug. On the other hand, direct toxicological effect of DSP up to 5.0 mg/kg may not necessarily be of major significance, considering the normal histology in the heart, lungs, liver or kidneys.

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Key words: DSP, histopathology, internal organs, thy 1, B220.

INTRODUCTION

(-)15-Deoxyspergualin (DSP) was originally developed in Japan (Takeuchi et al., 1981), and was shown to have a potent immunosuppressive effect in various models (Takeuchi et al., 1981; Ishizuka, 1986; Makino et al., 1987; Suzuki et al., 1987; Masaki et al., 1988; Okubo et al., 1988; Okubo et al., 1989). Prior to its clinical application, the accumulation of toxicological information may have to be complete. Since no data is yet available on its subacute toxicity in mice, this as well as the preceding report, which dealt with the hematological data of subacute toxicity, were designed to study the possible complications in mice given DSP for three months. The present report was focused on the histopathological aspects of the murine toxicology of DSP.

MATERIALS AND METHODS

Four-week-old female BALB/c mice were purchased from Japan SLC, Inc. (Shizuoka). The breeding conditions of the animals, study protocol, and the preparation and administration of DSP were identical with those described in detail in the preceding report.

Histopathological studies: The wet weights of heart, lungs, liver, spleen and kidneys were measured immediately following the sacrifice. Subsequently, portions of each were fixed with 10% neutral formalin, stained with hematoxylin-eosin (HE) or with periodic acid Schiff, and examined under light microscope. Another portion of spleen was embedded in OCT, snap-frozen with liquid nitrogen, and 4 µ-thick frozen sections were prepared with cryostat. After being fixed with acetone, they were successively incubated with monoclonal rat anti-mouse thy 1 (HO-13, a generous gift from Dr. Shirai, Juntendo University School of Medicine) or with anti-mouse B220 for 60 minutes, then with biotinated goat anti-rat IgG(Cappel Laboratories, Malvern, PA) for 45 minutes, and with avidin-biotin complex (Vector Co., Burlingame, CA) plus DAB solution (0.2 mg/ml DAB and 0.1 µl/ml H₂O₂ in 50 ml Tris buffer, pH 7.4). Lastly, the specimens were stained with HE, and examined under light microscope.

Statistical analyses were done using Student’s t-test. Data were expressed as mean ± SD throughout the text.

RESULTS

1. The wet weights of internal organs (Fig. 1): The mean weights of heart, lungs, liver, spleen and kidneys in the 0.5 mg- and 2.5 mg-DSP groups were not significantly different from those of the respective organs taken simultaneously from
the control animals, except the heart weight in the 0.5 mg-DSP group at day 45. On the other hand, the weights of the heart, lungs, liver and spleen in the 5 mg-DSP group of mice at day 23 and/or 38 were significantly lower compared with the control at day 0.

2. Histopathological findings of the internal organs (Table 1): No histopathological abnormalities were observed in heart, lungs, liver or kidneys taken from the animals given 0.5–5.0 mg/kg of DSP. In contrast, significant changes were seen in spleen and bone marrow of the 5 mg group of mice, and likewise, dose-dependent lesions were seen in the intestine of the 0.5–5.0 mg groups (Table 1). Atrophy of splenic corpuscles or white pulps, no clear demarcations between red and white pulps, decrease in the cell number of the red pulp and interstitial fibrosis were observed (Photo. 1). Decreased cells, accumulation of megakaryocytes and dilated sinusoids were seen in the sternal bone marrow (Photo. 2). On the other hand, there were degeneration and disappearance of the superficial mucosal cell layer and infiltration by inflammatory cells in the intestine (Photo. 3). In addition, there was pseudo-membrane formation in some of the mice treated with 5 mg/kg of DSP.

3. Immunohistological studies: Decrease in cell number observed in the spleen was further evaluated with avidin-biotin complex (ABC) technique. Decrease in B220\(^+\) cells in the splenic corpuscles seen in the DSP-treated mice as compared with the control mice was compatible with the atrophy of the corpuscles (Photo. 4). In contrast, decrease in T cells in the DSP-treated mice, which were mostly present along the arterial sheath, was found to be less conspicuous compared with the decrease in B cells mentioned above.

DISCUSSION

The effect of subacutely administered DSP on the wet weights of various internal organs was found to be dose-dependent. 0.5 mg group of mice showed no difference in the mean weights of the organs compared with the control mice, and 2.5 mg group showed only mild decrease of the body weight as well as of the spleen weight, together with the leukopenia. These changes due to 2.5 mg/kg DSP given to the mice were comparable with those seen in rats given 0.67 mg/kg DSP (Aoyagi et al.: unpublished observation). Accordingly, mice may be approximately 3.7 times more tolerant than rats against subacutely administered DSP. Five mg group of mice as well as 4 mg group of rats showed manifest decrease in body weight, leukopenia, anemia, thrombocytosis, decrease in spleen weight and mild decrease in the weight of heart, lungs and liver. In contrast, when dogs were given DSP, i. v., 0.15 mg/kg produced soft stool and mild pulmonary edema, while 0.3 mg/kg caused diarrhea, weight loss, anemic tendency, pulmonary edema, mucosal change in the colon, and dilated red marrow in the bone (Aoyagi et al.: personal communication).

Histopathological studies revealed no significant changes in heart, lungs, liver and kidneys. On the other hand, manifest lesions were observed in spleen, bone marrow
Fig. 1. The effect of 15-deoxyspergualin on wet weights of heart, lungs, liver, spleen and kidneys.

P values in the 0.5 mg- or 2.5 mg-DSP group were against the respective contemporary control groups, while those in the 5.0 mg-DSP group were versus the pretreatment level.
Table 1. Toxicological effect of DSP on histology* of spleen, bone marrow and intestine.

<table>
<thead>
<tr>
<th>Days on Tx</th>
<th>No of mice</th>
<th>Atrophy of White Pulp</th>
<th>Cellular Decrease in Red Pulp</th>
<th>Interstitial Fibrosis</th>
<th>Cellular Decrease</th>
<th>Dilatation of Sinusoid</th>
<th>Cellular Degeneration and/or Cell Infiltrations</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 mg-DSP group</td>
<td>23</td>
<td>9</td>
<td>1.3</td>
<td>1.5</td>
<td>0.9</td>
<td>n. s. b</td>
<td>1.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±0.57</td>
<td>±0.61</td>
<td>±0.50</td>
<td></td>
<td></td>
<td>±0.78</td>
</tr>
<tr>
<td>5 mg-DSP group</td>
<td>38</td>
<td>5</td>
<td>1.3</td>
<td>1.8</td>
<td>1.5</td>
<td>1.4</td>
<td>1.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±0.67</td>
<td>±0.45</td>
<td>±0.71</td>
<td>±0.55</td>
<td>±0.55</td>
<td>±0.84</td>
</tr>
<tr>
<td>2.5 mg-DSP group</td>
<td>57</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>±0.84</td>
</tr>
<tr>
<td>0.5 mg-DSP group</td>
<td>57</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.1</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>±0.32</td>
</tr>
<tr>
<td>Control group</td>
<td>57</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* Histological changes were classified as negative, very mild, mild, or severe changes, each being scored 0, 0.5, 1 or 2, respectively. Mean ± SD is shown in the Table.

b Not studied.
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Photo 1. Histopathology of the spleen in a mouse treated with 15-deoxyspergualin for 23 days.
   a: A control mouse; b: a mouse treated with 5.0 mg/kg DSP. PAS-staining.

Photo 2. Histopathology of the bone marrow in a mouse treated with 15-deoxyspergualin for 23 days.
   a: A control mouse; b: a mouse treated with 5.0 mg/kg DSP. PAS staining.
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Photo 3. Histopathology of the intestine in mice subacutely treated with 15-deoxyspergualin.

a: A control mouse ; b: a 0.5 mg DSP-treated mouse ; c: a 2.5 mg DSP-treated ; d: a 5.0 mg DSP-treated. Mice a, b and c were treated for 57 days, while mouse d was treated for 23 days. PAS staining.


a: A control mouse ; b: a 5 mg DSP-treated mouse. Stained with avidin-biotin complex system (see text)
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and intestine. Five mg/kg DSP produced atrophy of white pulp, blurred demarcation between white and red pulps, cellular decrease in red pulp, and interstitial fibrosis in the spleen. Likewise, cellular decrease and dilatation of sinusoid were seen in bone marrow. On the other hand, degenerative changes, defect and pseudo-membrane formation in the intestinal epithelium, and/or infiltration by inflammatory cells were observed in some of the 2.5 mg group of mice and in all of the 5.0 mg group of animals. These changes seen predominantly in the lymphoid and hematopoietic organs may be directly related to the potent immunosuppressive action inherent to this drug. To further investigate the latter possibility, the effect of DSP on spleen lymphocyte surface markers were studied by ABC technique. Preferential decrease in B cells rather than T cells was observed in the spleen of mice treated with DSP. In addition, DSP was shown to suppress plaque forming cell generation in vitro due to T-independent antigen (Okubo et al., 1989). These data suggest that direct action of DSP against B cells may at least partially be relevant to the decreased antibody production observed in mice treated with DSP (Okubo et al., 1988). Summarizing the toxicology of DSP, direct toxic effect against organs of this drug up to 5.0 mg/kg may not necessarily be of major significance, taking into account the normal histology in the heart, lungs, liver or kidneys taken from animals given this dose. However, considering the manifest effect on lymphoid and hematopoietic organs as well as leukopenia and anemia seen in the 5.0 mg-DSP group, the safety limit of the long-term treatment of mice with DSP may be presumed to be 2.5 mg/kg body weight or less.

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REFERENCES


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