SUBACUTE TOXICITY OF (-)15-DEOXYSPERGUALIN IN BALB/c MICE. I. HEMATOLOGICAL STUDY

Michihito OKUBO, Yoshihiko MASAKI*, Keiichi INOUE,
Rieko HARA**, Miyako TAIRA**, Kouju KAMATA
and Huiming ZHANG***

Departments of Medicine, *Experimental Animals, **Clinical Pathology and ***Pathology,
Kitasato University School of Medicine,
1-15-1 Kitasato, Sagamihara-shi, Kanagawa 228, Japan

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Abstract.....(-)15-Deoxyspergualin (DSP), a bacterial metabolite obtained from *Bacillus laterosporus*, was shown to effectively suppress autoantibody production in mice and alloimmune reaction in mice and rats. Its potential toxicity and safety margin in BALB/c mice were studied by administering the drug, 0.5–5.0 mg/kg body weight, s. c., 6 days/week for 3 months. The animals were weighed every two weeks, and their blood was drawn for hematological and liver function studies.

Control animals showed a gradual increase in body weight from 17.8 ± 1.1 g (SD) to 20.2 ± 3.2 g during the 3 months of treatment. The body weight of 0.5 mg- and 2.5 mg-DSP group was not significantly different from that of the control group. In contrast, 5.0 mg-DSP group of animals showed significant decrease in their weight compared with the control after 5 weeks of treatment ($P < 0.01$). They were then sacrificed. WBC in 0.5 mg-, 2.5 mg- and 5.0 mg-DSP groups were significantly lower compared with the control after 3 months of treatment. On the other hand, only 5.0 mg-DSP group showed significantly lower level in their RBC, Hb and Hct values ($P < 0.05$). In contrast, platelet counts in 2.5 mg- and 5.0 mg-DSP groups significantly increased. No manifest effect due to DSP on differential count of leukocytes was observed, although on day 92, a significant difference was shown in the stab cells of the 0.5 mg-DSP group and in the eosinophils in the 2.5 mg-DSP. SGOT level following 5.0 mg-DSP treatment for 5 weeks was significantly elevated compared with the pre-treatment level ($P < 0.05$). The present study shows that the safety limit of long-term treatment with DSP may be 2.5 mg/kg body weight or less and that DSP preferentially affects lymphoid organs, although higher dose of DSP may also be toxic to the bone marrow system.

Correspondence : Dr. Michihito OKUBO at the above address.

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INTRODUCTION

(-)-15-Deoxyspergualin (DSP) was originally developed as an anti-tumor antibiotic, and was found to show an anti-tumor activity against mouse leukemic cell line L-1210 (Takeuchi et al., 1981). Its chemical structure was determined to be (-)-(15S)-1-amino-19-guanidino-11, 15-dihydroxy-4, 9, 12-triazanonadecane-10, 13-dione (Umezawa et al., 1981) (Fig. 1). A potent immunosuppressive effect of DSP was subsequently shown (Ishizuka, 1986), stimulating a number of *in vitro* as well as *in vivo* studies in various disease categories. We already reported that administration of DSP markedly prolonged the life span and inhibited the development of glomerulonephritis in New Zealand Black / White F1 mice (Okubo et al., 1988). The survival of thyroid allografts in mice was likewise significantly prolonged by DSP (Masaki et al., 1988).

Since clinical applicability of a drug depends on its safety margin as well as on its effectiveness, strict monitoring of the possible complications inherent to the drug may be of prime importance. No data is yet available on subacute toxicity produced by DSP in mice, although it was studied in rats and dogs (Aoyagi et al., unpublished data). Accordingly, DSP was subcutaneously injected in mice for three months to measure the safety limit as well as to study the possible subacute toxicity of the drug in this animal model.

15-Deoxyspergualin

Fig. 1. Structure of 15-deoxyspergualin.

MATERIALS AND METHODS

Experimental animals : Five-week-old female BALB/c mice were purchased from Japan SLC, Inc. (Shizuoka), and kept for one week in our mouse unit before use. Ninety-two mice were divided into four groups : thirty animals given 0.05 ml

PBS only, twenty mice given DSP 0.5 mg/kg body weight, twenty-two given DSP 2.5 mg/kg, and the remaining twenty given DSP 5.0 mg/kg. Breeding conditions in our mouse units were as follows : temperature $25 \pm 1^\circ\text{C}$, moisture $45 \pm 5\%$, in all fresh air system, and twelve hours each of white light and darkness due to semi-barrier system. Five to six mice were housed in a transparent cage ($300 \times 210 \times 125$ mm, Clea Japan, Inc., Tokyo). They were given solid food (CE-2, Oriental & Co., Tokyo) and tap water *ad lib*.

Preparation and administration of DSP : DSP was dissolved at 2 mg/ml in PBS, divided into aliquots, and kept frozen at -20°C in 2-ml sample tubes until used. Mice were subcutaneously given PBS 0.05 ml, DSP 0.5 mg/kg, 2.5 mg/kg or 5.0 mg/kg six days per week for three months. These doses were chosen since New Zealand Black / White F1 mice well tolerated up to 6 mg/kg of DSP, four times per week, or approximately 3.4 mg/kg/day, given since 14 weeks of age until their natural death, although slight but significant decrease in the mean body weight was observed in those mice given the highest dose (Okubo et al., 1988). Animals were daily checked for their general appearance, behaviour, hair, stool, and appetite, and were weighed every two weeks. Five mg/kg DSP group of mice were sacrificed on day 23 or 38 because of anorexia, progressive weight loss and aggravation of general condition.

Hematological examination : Animals were decapitated prior to, two months or three months following the start of the DSP treatment. Their blood gushing out of the carotid arteries was collected into glass tubes with EDTA, and the white and red cell counts, hematocrit, hemoglobin level and platelet counts were determined with autoanalyzer (Sysmex E-4000, Toa Medical Electronics, Inc., Kobe). The differential counts of leukocytes were measured by examining Giemsa-stained preparation under light microscopy.

SGOT and SGPT were measured with microautoanalyzer (HITACHI 736-50, Hitachi, Ltd., Tokyo).

The wet weight of various internal organs, such as heart, lungs, liver, spleen and kidneys, and *the results of histological examinations* will appear in the succeeding report in the same issue.

Statistical analyses were done using Student's *t*-test. Data were expressed as mean $\pm$ SD throughout the text.

RESULTS

1. Overall clinical condition : No mice belonging to the control, 0.5 mg-DSP or 2.5 mg-DSP group was dead during the three months of study for subacute toxicity. In contrast, 5.0 mg-DSP group of mice showed anorexia and decrease in body weight since two weeks after start of the DSP administration, and five out of the ten mice were dead by four weeks or so of treatment. Since marked weight loss was observed in the remaining five mice, they were sacrificed and studied at five weeks.

2. Body weight : The mean body weight of the control group at start of the study was 17.8 ± 1.10 g (Fig. 2). The control and 0.5 mg-DSP groups showed gradual increase in their body weight during the three months of study period, and their mean weights after three months were 20.2 ± 3.20 g and 21.9 ± 1.39 g, respectively. On the other hand, the mean body weight of the 2.5 mg-DSP group at eight weeks was significantly lower compared with that of control group ($P < 0.05$), although at thirteen weeks their weight (18.3 ± 3.85 g) was not different compared with the control. Five mg-DSP group showed a marked decrease in their body weight, which was 12.9 ± 1.59 g at day 38 prior to termination of the study, and was significantly lower compared with any other groups of mice ($P < 0.01$).

Fig. 2. Variations in the body weight in mice subacutely treated with 15-deoxyspergualin. P values in the 0.5 mg- or 2.5 mg-DSP group were against their respective contemporary control groups, while those in the 5.0 mg-DSP group were versus the pre-treatment level.

3. Hematological study : White blood cell counts in the DSP-treated groups at 5 weeks (5 mg-DSP group), 8 weeks (0.5 mg- and 2.5 mg-DSP groups) or 12 weeks (2.5 mg-DSP group) were significantly lower than those in their respective controls (Fig. 3). Likewise, red blood cell counts in 0.5 mg- and 2.5 mg-DSP groups at 13 weeks were significantly decreased. The hemoglobin and hematocrit levels in 0.5 mg- and 2.5 mg-DSP groups were not different compared with those in the control group, although the levels in the 5 mg-DSP group were markedly decreased. On the other hand, platelet counts in the 2.5 mg-DSP group at 8 weeks and in 5 mg-DSP group at 3 and 5 weeks showed an increase compared with the control and the pre-treatment level, respectively (Fig. 4).

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Fig. 3. The effect of 15-deoxyspergualin on WBC, RBC, hemoglobin and hematocrit. P-values in the 0.5 mg- or 2.5 mg-DSP group were against their respective contemporary control groups, while those in the 5.0 mg-DSP group were versus the pre-treatment level.

Fig. 4. The effect of 15-deoxyspergualin on the platelet count.
See the legend of Fig. 3 for P values.

No manifest effect due to DSP was seen in the leukocyte differential counts, although at day 93 the stab cells in the 0.5 mg-DSP group and eosinophils in the 2.5 mg-DSP group showed a significant difference compared with their respective controls (Table 1).

4. Serum transaminase : The levels of SGPT and SGOT in the 0.5 mg- and 2.5 mg-DSP groups both at days 57 and 93 were not different compared with the control (Fig. 5). In contrast, SGOT in the 5.0 mg-DSP group at day 28 was significantly higher than the control level at day 0 ($P<0.05$).

DISCUSSION

The mean body weight in 0.5 mg- or 2.5 mg-DSP group of mice was not different from that in the control group, while 5 mg-DSP group rapidly lost their weight compared with the control group at day 0. Accordingly, the clinically tolerable maximal dose of DSP given subacutely may be assumed to be around 2.5 mg/kg. The doses of 2.5 mg/kg or higher may suppress the bone marrow, since leukocytopenia and anemia were observed at 2.5 mg and 5 mg/kg. In contrast, platelet count rather increased at these doses. The genesis of thrombocytosis due to

Table 1. Hemograms in mice subacutely treated with 15-deoxyspergualin.

	0	23	38	57	93	days
Band	①	2.8±1.7		6.0±1.6**	8.8±4.9**	#
	②			7.0±1.2**	3.5±1.9	
	③			9.0±2.4**	5.0±3.0	
	④		3.4±2.3			
Seg	①	19.8±4.7		29.8±11.7	33.0±6.3**	
	②			35.8±5.9**	27.7±8.0	
	③			16.0±8.4	26.6±2.9*	
	④		12.6±3.9	26.8±13.1		
Eosin	①	6.4±1.1		2.6±1.5	2.6±0.9**	#
	②			3.2±1.3	2.5±2.3**	
	③			2.5±1.3**	6.0±2.6	
	④		0			
Lymph	①	71.2±5.1		59.8±9.1*	56.2±10.3**	
	②			53.2±7.6**	65.0±9.4	
	③			72.2±10.4	63.2±6.8	
	④		83.4±5.9	68.0±11.2		
Mono	①	1.8±1.0		1.8±0.8	3.0±2.3	
	②			2.8±2.0	1.5±0.5	
	③			1.8±0.5	2.0±0.8	
	④		1.0±0	2.6±1.5		

① : control ② : DSP 0.5mg/kg ③ : DSP 2.5mg/kg ④ : DSP 5 mg/kg

** P<0.01 * P<0.05 V control before tx.

P<0.05 V respective control.

Fig. 5. The effect of 15-deoxyspergulin on serum GPT and GOT.
P value was versus the pre-treatment level.

DSP is not known at present, although the same phenomenon was also observed in dogs (see the next paragraph).

According to Aoyagi et al (unpublished data), 0.67 mg/kg of DSP given, i. p., to rats caused mild leukocytopenia and anemia on day 91 as well as mild thrombocytosis. In addition, slight thymic atrophy was observed by the pathological study. Weight increase was suppressed in male animals at the 6th week of DSP administration or later. In the 4.0 mg-DSP group, the above findings were more marked, and the decrease in body weight since the 2nd week, severe leukocytopenia, anemia, and liver dysfunction were observed. The decrease in weight and atrophy of thymus, liver and testicles were likewise seen. Since the 2.5 mg/kg DSP administered to mice in the present study caused mild decrease in body weight, leukocytopenia, and mild transient thrombocytosis, this dose may be nearly equivalent to the dose of 0.67 mg/kg in the rat, that is DSP being likely to be 3.7 times less toxic in mice. Five mg-DSP group of mice as well as 4.0 mg-DSP group of rats showed marked decrease in body weight, leukocytopenia, anemia,

thrombocytosis, decrease in spleen weight, and more mild decrease in the weight of heart and liver (as for the data on organ weights, see the succeeding report in the same issue). However, neutrophil / lymphocyte ratio in the differential count was not affected, and decrease in granulocyte counts was not observed. Due to the above unpublished report, 0.15 mg/kg of DSP given to the dogs caused soft stool and mild pulmonary congestion was observed at autopsy, while 0.3 mg DSP caused diarrhea, decrease in body weight, anemic tendency, pulmonary edema, mucosal change in the colon, and dilated red pulp in the bone marrow. Summarizing the above data, it may be said that various complications such as decrease in body weight, diarrhea, suppressive effects on hematopoietic system like leukocytopenia and anemia, and liver dysfunction may be inherent to DSP administration, in addition to various pathological changes observed in spleen, intestine and liver. Although no data in mice is available, kidneys may be considered to be the major route of excretion of DSP at least in rats, since following i. v. injection of ^3H - and ^{14}C -labelled DSP, almost all of the radioactivity was collected in the urine within 24 hours (Aoyagi et al., unpublished data). No deleterious effects of DSP on kidneys have been observed except a rise in BUN seen in rats given a large dose of DSP. Because of the dose-dependency of the above complications, determination of safety limit and optimal dose in different species as well as the efficacy of the combination therapy with other drugs remain to be clarified.

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