Note

SUPPRESSION OF EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS IN GUINEA PIGS BY SPERGUALIN AND 15-DEOXYSPERGUALIN

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Spergualin (SGL) is a metabolite of *Bacillus laterosporus*[1]. 15-Deoxyspergualin (DSG) is an analogue of SGL. Both compounds have strong immunosuppressive activities in animals[2~4]. In separate publications[3~5], DSG was proved to prolong allograft survival in many experimental transplantation models. However, it has yet to be shown whether both compounds are valuable as immunosuppressive agents in models of autoimmune disease. The results presented here demonstrate the efficacy of these compounds on experimental allergic encephalomyelitis (EAE) in guinea pigs.

SGL and DSG were prepared at Takara Shuzo Co., Ltd., dissolved in saline, sterilized by passing through a 0.22-μm filter and stored at $-20°C$ until use. Inbred male strain 13 guinea pigs (350~850 g) were purchased from Nisseiken, Tokyo, Japan. EAE was induced as described below. An aqueous suspension of 50% homogenate of the spinal cord obtained from guinea pigs was emulsified in an equal volume of complete FREUND's adjuvant containing *Mycobacterium tuberculosis* H37 Ra (Difco) at 20 mg/ml. The antigen emulsion (0.1 ml) was injected sc into both hind footpads of the experimental animals. Data were analyzed by Student's t-test.

† Deceased.

Table 1. Effect of SGL on development of EAE.

SGL (mg/kg)	Grade of clinical sign by day 13 (score)	Mean survival time (days)
0	3.0±0.0	13.2±0.8
1.56	2.2±1.1	15.0±1.4*
3.13	1.6±1.3	15.2±1.3*
6.25	0.4±0.9	17.4±1.1**
12.5	0.0±0.0	16.8±1.6**

SGL was administered ip once a day for 4 days starting 1 day after the immunization. Thirteen days later the animals were observed for clinical signs: 0; no sign, 1; hind paraparesis or incontinence, 2; hind paraparesis accompanied with incontinence, and 3; quadriparesis or death. Each group consisted of 5 animals. All data are shown as mean with SD.

* $P<0.05$.

** $P<0.01$.

Table 2. Prolongation of life span in the guinea pigs induced EAE by SGL and DSG administrations for 2 weeks.

Agent	Dose (mg/kg)	Survival time (days)	Significance
Saline		13, 13, 15, 16, 16	
SGL	0.78	13, 15, 15, 16, 17	
	1.56	19, 19, 20, 20, >40	$P<0.05$
Saline		13, 13, 13, 15, 15	
DSG	1.56	17, >40, >40, >40, >40	$P<0.001$

Agents were administered ip once a day for 14 days starting 1 day after the immunization. Each group consisted of 5 animals.

Guinea pigs which were immunized without receiving an immunosuppressant died within 14 days (Table 1). These animals showed severe symptoms of EAE such as paralysis and incontinence. When SGL was administered daily at doses of 1.56 to 12.5 mg/kg for 4 days starting 1 day after immunization, a marked suppression of the appearance of distinct symptoms and a significant prolongation of mean survival time

were observed. The effects were maximal at the higher doses of 6.25 and 12.5 mg/kg, but the all animals died with EAE symptoms within 20 days.

Since slight diarrhea and weight loss were observed in the animals treated with SGL at doses over 6.25 mg/kg, a second series of experiments were carried out using the lower doses of 0.78 and 1.56 mg/kg. The administration period was prolonged for 2 weeks. Once again SGL at a dose of 1.56 mg/kg significantly prolonged the survival time of immunized animals (Table 2). DSG, which has shown a stronger activity than SGL in inhibiting the rejection of skin allografts in rats[3], was administered at the same dose of 1.56 mg/kg following the same administration schedule. A significant prolongation of the survival time was found and these animals (4 out of 5) survived without the symptoms of EAE. At these doses the SGL and DSG-treated animals did not show the diarrhea and weight loss observed in the earlier experiments. No other toxic symptoms were observed.

In the present study, SGL and DSG were found to be prophylactically effective on EAE. DSG was more effective than SGL, when given at a dose of 1.56 mg/kg for 14 days. It requires further study to see whether DSG has a beneficial effect on EAE, when given therapeutically.

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