A NEW ANTITUMOR ANTIBIOTIC, SPERGUALIN: ISOLATION AND ANTITUMOR ACTIVITY

Sir:

In testing the activity of culture filtrates to inhibit focus formation of chicken fibroblasts by Rous sarcoma virus, an antibiotic produced by a bacillus isolated from a soil sample collected at Ohira-san, Tochigi Prefecture was thought to be worthy of further study, even though daily intraperitoneal injection (0.25 ml) of its culture filtrate for 9 days caused death of mice and showed no effect in prolonging the survival period of mice bearing L-1210 leukemia. Since the culture filtrate inhibited the growth of *B. subtilis* and *S. aureus*, we extracted and purified the antibacterial activity. The antibiotic thus purified had a low toxicity and exhibited a marked effect against mouse L-1210 leukemia. The ratio of 50% inhibition concentration (13 μg/ml) against the growth of chicken fibroblasts to 50% inhibition concentration (5.5 μg/ml) against focus formation by Rous sarcoma virus was 2.3. On the basis of the structure which will be reported in the next paper[1], this antibiotic was named spergualin. In this paper, we will report on the isolation and antitumor activity of spergualin.

The strain producing spergualin was numbered as BMG162-aF2 in the authors' Institute. The taxonomic study suggested that this strain was closely related to *Bacillus laterosporus*. Spergualin was produced, extracted and purified by the following processes. The strain BMG162-aF2 was shake-cultured at 28°C in a medium containing 2.0% glycerol, 2.0% dextrin, 1.0% soy peptone (Bacto-soytone, Difco Laboratories), 0.3% yeast extract, 0.2% $(NH_4)_2SO_4$ and 0.2% $CaCO_3$ (adjusted to pH 7.4). After 3~4 days culture, more than 100 μg/ml of spergualin was produced as shown by a cylinder plate assay using *Bacillus subtilis* PCI219 as the test organism. The antibiotic in the culture filtrate (4,900 ml) was adsorbed on a column (500 ml in 5.2 cm diameter) of Amberlite IRC-50 (70% Na^+ form) and eluted with 1 N HCl (2,000 ml). After neutralization of the eluate, the antibiotic was adsorbed on a column (400 ml in 4.3 cm diameter) of CM-Sephadex C-25 and eluted with 0.3 M NaCl. The active eluate was concentrated to dryness and the residue was extracted with methanol (5 ml). The methanol solution was passed through a column (445 ml in 2.6 cm diameter) of Sephadex LH-20 and the column was developed with methanol. The concentration of the active fractions gave pure spergualin trihydrochloride as a colorless hygroscopic powder (460 mg). Physicochemical properties are described in the next paper[1].

Spergualin trihydrochloride is easily soluble in water and as shown in Table 1, in the usual agar dilution method on a nutrient agar plate, spergualin at 50~100 μg/ml inhibited the growth of Gram-positive and -negative bacteria except *Serratia marcescens* and *Ps. aeruginosa*. *B. subtilis* PCI219, *S. aureus* Smith, *Sal. typhi* T-63 and *Pr. vulgaris* OX19 were relatively sensitive to spergualin and their growth was inhibited at 6.25 μg/ml.

In a test where 10^5 L-1210 cells were intraperitoneally inoculated and spergualin dissolved in saline was injected daily from the following day

Table 1. The antimicrobial spectrum of spergualin trihydrochloride.

Test organisms	Minimum inhibitory concentrations (μg/ml)
Staphylococcus aureus FDA209P	50
Staphylococcus aureus Smith	6.25
Micrococcus flavus FDA16	25
Micrococcus luteus PCI1001	50
Bacillus anthracis	12.5
Bacillus subtilis PCI219	6.25
Bacillus subtilis NRRL B-558	25
Bacillus cereus ATCC10702	50
Corynebacterium bovis 1810	50
Escherichia coli NIHJ	25
Escherichia coli K-12	50
Escherichia coli K-12 ML1629	50
Escherichia coli K-12 ML1630	50
Klebsiella pneumoniae PCI602	50
Shigella dysenteriae JS11910	50
Shigella flexneri 4b JS11811	100
Shigella sonnei JS11746	50
Salmonella typhi T-63	6.25
Salmonella enteritidis 1891	100
Proteus vulgaris OX19	6.25
Serratia marcescens	>100
Pseudomonas aeruginosa A3	>100
Pseudomonas aeruginosa No. 12	>100

Table 2. Antitumor effect of spergualin on L-1210.

Schedule (day)	Dose (mg/kg/day)	T/C (%)	Survivor (60 days)
1~9 (i.p.-i.p.)	50	295	0 / 8
	25	334	0 / 8
	12.5	586	4 / 8
	6.25	732	8 / 8
	3.13	441	3 / 8
	1.56	301	1 / 8
	0.78	107	0 / 4

Inoculum size: 10^5 cells/mouse.

Table 3. Antitumor effect of spergualin on L-1210 solid type.

Schedule (day)	Dose (mg/kg/day)	T/C (%)	Survivor (30 days)
1~9 (s.c.-i.p.)	50	>309	5 / 5
	25	>309	5 / 5
	12.5	>309	5 / 5
	6.25	>240	3 / 5
	3.13	120	0 / 5
	1.56	105	0 / 5

Inoculum size: 10^5 cells/mouse.

the tumor cell inoculation, for 9 days, as shown in Table 2, 1.56~50 μg/mg/day prolonged the survival period of mice markedly. All mice treated with spergualin 6.25 mg/kg/day survived. The control mice died 7.5~8.5 days after the tumor cell inoculation. The death of mice treated with 25 or 50 mg/kg/day was not due to the toxicity of spergualin. This was due to L-1210 leukemia, because the tumor cells grew as ascites before death. Mice died 7~33 days after the last injection of spergualin. As will be reported in an other paper, the inoculation of the tumor cells taken from mice treated with 50 μg/mouse was significantly more resistant to spergualin treatment than the inoculation of the original L-1210 cells.

As shown in Table 3, spergualin showed a marked effect in prolonging the survival of mice to which 10^5 L-1210 cells were inoculated subcutaneously. All mice treated by intraperitoneal injection of spergualin 12.5, 25, 50 mg/kg daily survived. The treatment was started 1 day after the tumor cell inoculation and continued for 9 days. The mice without treatment died 8~10 days after the inoculation. The mice which survived were resistant to the second inoculation of L-1210 cells.

Table 4. Antitumor effect of spergualin on EL-4 mouse leukemia.

Schedule (day)	Dose (mg/kg/day)	T/C (%)
1~9 (i.p.-i.p.)	5	193
	2.5	164
	1.25	159
	0.625	130
	0.313	131

Inoculum size: 10^5 cells/mouse.

Spergualin also prolonged the survival period of mice to which 10^5 cells of EL-4 mouse leukemia were inoculated as shown in Table 4.

Spergualin also prolonged the survival period of mice bearing Ehrlich carcinoma in the ascites form: 2×10^6 Ehrlich carcinoma cells were inoculated intraperitoneally to mice; spergualin was given intraperitoneally daily from 1 day after the inoculation for 9 days, and the percentage of the survival days to those of the control was 133% by 1.56 mg/kg/day, 188% by 3.13 mg/kg/day, 236% by 6.25 mg/kg, 170% by 12.5 mg/kg. Higher doses such as 25 or 50 mg/kg/day shortened the survival period (72% by 25 mg/kg/day, 54% by 50 mg/kg/day). In this case, ascites did not increase and the cause of the death was not certain.

In experiments performed by a procedure similar to the one to test the effect against Ehrlich carcinoma, spergualin prolonged the survival period of mice bearing sarcoma 180: 220% by 1.56 mg/kg/day, 243% by 3.13 mg/kg/day, 181% by 6.25 mg/kg/day, 279% by 12.5 mg/kg/day. High doses such as 50 mg/kg/day or 25 mg/kg/day shortened the survival period (53% by 50 mg/kg/day; 69% by 25 mg/kg/day).

Spergualin has low toxicity: intravenous injection of 80 mg/kg did not cause the death of mice but the LD_{50} markedly fluctuated depending on the injection speed. The LD_{50} by intraperitoneal injection was about 150 mg/kg. It was found that spergualin seems to have no cumulative toxicity.

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