ANTITUMOR ACTIVITY OF LEPTOMYCIN B

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(Received for publication December 3, 1984)

Leptomycin B was first reported by HAMAMOTO *et al.* as an antifungal antibiotic, and showed remarkable cytotoxicity on mammalian cells[1]. Thereafter, we also isolated this antibiotic from the fermentation broth of *Streptomyces* sp. No. 81-484 as a byproduct of kazusamycin. In the previous paper[2,3], we reported that kazusamycin and leptomycin B are similar in their physicochemical and biological properties. Since kazusamycin shows antitumor activities on experimental tumors[2,4], we tested leptomycin B for antitumor effects on transplantable murine tumors.

Leptomycin B was isolated and purified according to the same procedure reported previously[2,3]. Tumors were inoculated intraperitoneally into mice (6 weeks old). Leptomycin B was dissolved in a small amount of MeOH and Tween-80 and diluted with saline, and injected intraperitoneally into tumor bearing mice according to the schedules shown in Table 1. Antitumor activity was evaluated by the increase in life span (ILS): $(T/C-1)\times 100\%$, where "T" is the mean survival days (MSD) of the treated group and "C" is the MSD of the control group. Survival of mice was scored 60 days after implantation of tumors, and mice remaining alive after this period of observation were considered cured.

Antitumor activity of leptomycin B on four different tumors is shown in Table 1. When leptomycin B was administered on days 1~5 at a dose of 0.16 mg/kg into mice bearing Ehrlich ascites tumors, two out of five mice were cured.

Using the same schedule, the maximum ILS was 102% for Lewis lung carcinoma, whereas the effect was slight on B-16 melanoma and P388 lymphatic leukemia.

To determine the cytotoxicity of leptomycin

Table 1. Antitumor activity of leptomycin B on murine tumors.

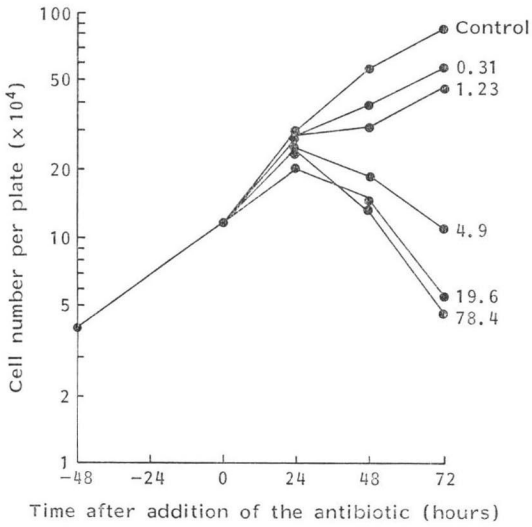
Treatment schedule	Total dose (mg/kg)	Increase in life span (%)			
		P388	Lewis lung	B16 melanoma	Ehrlich
Saline	—	0	0	0	0
Day 1	1.25		−31	0	36
	0.625	19	−3	0	23
	0.313	14	21	6	63
	0.156	13			39 (1)
	0.078				14
Days 1, 5, 9	1.25	23	−13	6	114 (1)
	0.625	23	34	11	49 (1)
	0.313	19	13	24	60
	0.156	11	30	15	55 (1)
	0.078	14			72
	0.039				24
Days 1~5	0.625	−45			−4
	0.313	4	−42	−18	97 (2)
	0.156	47	102	14	125 (1)
	0.078	40	48	32	53 (1)
	0.039	19	52	43	30

Tumor: P388 leukemia 1×10^5 cells/CDF_1, Lewis lung carcinoma 1×10^6 cells/C57BL, B16 melanoma 1×10^6 cells/C57BL, and Ehrlich carcinoma 2.5×10^6 cells/*dd*Y.

Mean survival days (range) of controls were as follows; P388 leukemia 10.6 (10~11), Lewis lung carcinoma 12.2 (11~14), B16 melanoma 22.8 (19~28), and Ehrlich carcinoma 21.1 (15~29).

Numbers in parenthesis indicate number of cured mice/five treated mice. Cured mice were excluded from the calculation of ILS.

Fig. 1. Effect of leptomycin B on HeLa cells *in vitro*.
Numbers in figure indicate concentration (ng/ml) of leptomycin B.



B, HeLa S3 cells (4×10^4 cells) in 2 ml of the medium [EAGLE's minimum essential medium supplemented with 10% calf serum and kanamycin (100 μg/ml)] were placed in a 2-cm^2 Petri dish (Falcon 3047, 24-well) and incubated for 48 hours at 37°C in a 5% CO_2 - 95% air atmosphere. Each culture dish was filled with fresh medium containing a different concentration of leptomycin B, and the incubation was continued for 24, 48 or 72 hours. HeLa cells were then trypsinized to form a single cell suspension, and counted in a hemocytometer.

Leptomycin B inhibited the growth of HeLa cells at a concentration of 4.9 ng/ml when the cells were exposed for three days (Fig. 1).

For morphological studies, HeLa cells (1×10^5) were plated in Leighton tubes each containing a coverslip. After 48 hours of cultivation at 37°C, the antibiotic dissolved in the growth medium was added to the tube, and the cells were reincubated for a further 48 to 96 hours. Morphological changes of the cells were observed microscopically after fixation and staining with Giemsa solution.

When HeLa cells were exposed to leptomycin B for 3 days, many polynuclear giant cells and masses of small nuclei appeared at a concentration of 2.5~1.25 ng/ml (Fig. 2).

Since leptomycin B showed strong cytotoxicity *in vitro* as indicated in the previous report and present experiment, it is considered that antitumor activity of the antibiotic is due mainly to direct cytotoxic activity on tumor cells.

Acknowledgment

We wish to thank Miss S. TAKANO for her technical assistance. This work was supported by a Grant-in-Aid from the Ministry of Health and Welfare, Japan, and Warner-Lambert Grant.

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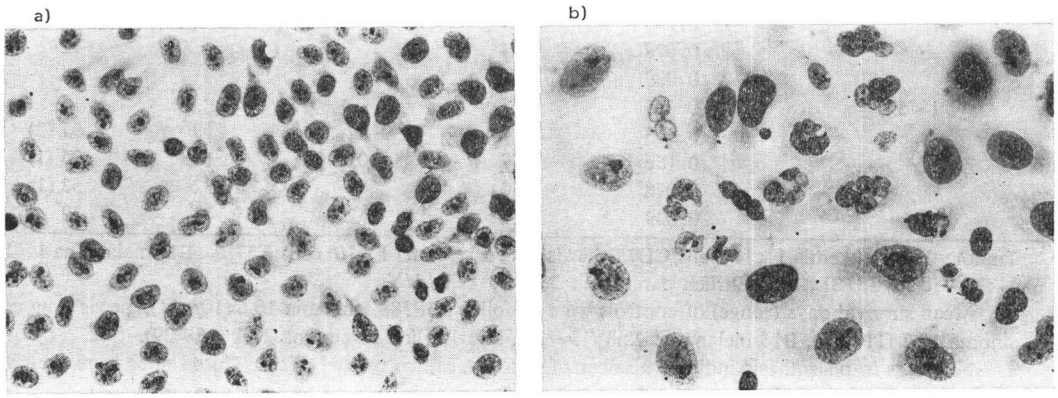
Fig. 2. Morphological change of HeLa cells exposed by leptomycin B.
a: Normal HeLa cells, b: HeLa cells exposed to 1.25 ng/ml of leptomycin B for 72 hours.



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