Communications to the editor

REVERSIBLE BINDING OF MACROMOMYCIN, A MACROMOLECULAR PEPTIDE ANTIBIOTIC, TO CELL MEMBRANES

Sir:

A new antitumor antibiotic macromomycin was isolated by CHIMURA *et al.*[1] from culture filtrates of *Streptomyces macromomyceticus*. It is a weakly basic protein having molecular weight of 15,000 and exhibits inhibitory action against mouse leukemia L-1210, mouse sarcoma 180 and various gram-positive bacteria. A number of macromolecular antitumor antibiotics have been isolated, however, their modes of action have not been studied extensively. The study of neocarzinostatin by ONO *et al.*[2,3] is one of the few well-studied cases. Considering their macromolecular nature, it is probable that some of these compounds exert their biochemical effects without penetrating target cells. This mechanism requires that simple binding of such macromolecules to the cell membrane freezes or modifies the function of the membrane, thereby affecting cell metabolism.

As reported in this communication, macromomycin apparently works in this manner. This conclusion was derived from the observation that the cytotoxicity of macromomycin is abolished by removal of this antibiotic from cells by brief treatment of the macromomycin-cell complex with trypsin.

YOSHIDA sarcoma cells were first incubated with ^{131}I-macromomycin (prepared by the method of McFARLANE[4] and HELMKAMP *et al.*[5] using ICl) for 35 minutes at 37°C. After washing with cold phosphate-buffered saline, the cells were treated with typsin and the suspensions chilled and centrifuged. The radioactivity in the supernatant solutions were determined. As shown in Table 1, trypsin at a concentration of 0.08 % released 84.3 % of the radioactivity which had been bound to the cells. Another experiment was conducted to determine if the cells could escape from the cytotoxic effect of the antibiotic under these conditions.

YOSHIDA sarcoma cells were treated with

Tabie 1. Release of ^{131}I-labeled macromomycin adsorbed to YOSHIDA sarcoma cells with trypsin

		cpm	Released %
Before incubation		11,280*	
After incubation with	buffer	6,920	38.6
	trypsin 0.03 %	3,640	66.8
	0.08 %	1,760	84.3

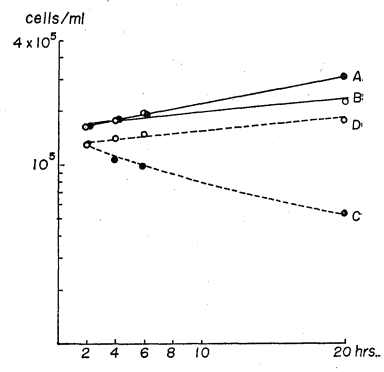
YOSHIDA sarcoma cells were taken from the peritoneal cavity of a rat 4 days after intraperitoneal inoculation of 2×10^6 cells. The cells were suspended in lactoalbumin-bovine serum (10 %) medium at the cell density of 2×10^7 cells/ml and incubated with 4 μg/ml of ^{131}I-macromomycin. After five washings with cold phosphate-buffered saline, the cells were resuspended at a cell density of 7.6×10^6 cells/ml in phosphate-buffered saline containing the indicated amount of trypsin. The suspensions were incubated for 10 minutes at 37°C, chilled and centrifuged. The radioactivity released into the supernatant solutions were determined and from these values the radioactivity remaining bound to cells was calculated. The cell-bound radioactivity (cpm) per 7.6×10^6 cells is shown.

* This value indicates that 5.2×10^4 molecules of macromomycin bound to a single cell.

Fig. 1. Effect of trypsin on the activity of macromomycin adsorbed to YOSHIDA sarcoma cells

YOSHIDA sarcoma cells (2.4×10^5 cells/ml in lactoalbumin-bovine serum medium) were treated with 4 μg/ml of macromomycin for 20 minutes at 37°C (1st incubation). After 3 washings with phosphate-buffered saline, cells were resuspended in phosphate-buffered saline containing 0.08 % trypsin and incubated for 9 minutes at 37°C (2nd incubation). After removal of trypsin by centrifugation, cells were incubated in fresh medium and numbers of viable cells were determined at the times indicated. Cell viabilities were determined in a hemocytemeter by exclusion of nigrosin.

A 1st incubation without macromomycin and 2nd incubation without trypsin
B 1st incubation without macromomycin and 2nd incubation with trypsin
C 1st incubation with macromomycin and 2nd incubation without trypsin
D 1st incubation with macromomycin and 2nd incubtion with trypsin



macromomycin for 20 minutes at 37°C. The cells were centrifuged and afterwards incubated in trypsin solution (0.08 %). After removal of the enzyme by centrifugation, the cells were incubated in a fresh medium. Samples were withdrawn at times indicated in Fig. 1 to count the numbers of viable cells. As shown in the figure, the trypsin digestion saved the cells from the lethal effect of the antibiotic, while a simple washing with phosphate-buffered saline did not.

It can be suggested thet mild treatment with trypsin modifies either the structure of the cell surface or the macromomycin molecule to interfere with binding.

In another experiment, the effect of macromomycin on the uptake of ^{3}H-thymidine, ^{3}H-uridine, ^{14}C-leucine into the acid-insoluble fraction of YOSHIDA sarcoma cells was examined. As shown in Table 2, DNA synthesis was appreciably reduced over a wide range of macromomycin concentrations, 2~50 μg/ml, RNA and protein syntheses were not inhibited even at 50 μg/ml, the highest concentration tested. It should be noticed, however, that the extent of inhibition of DNA synthesis did not exceed a maximum level of about 50 %.

As shown in the previous experiment (Fig. 1) 4 μg/ml of macromomycin was lethal to YOSHIDA sarcoma cells. This concentration must be high enough to inhibit DNA synthesis from the observation shown in Table 1. The lethal effect caused by macromomycin at this concentration was abolished by washing these cells with trypsin which released the bound macromomycin. These observations suggest that inhibition of DNA synthesis is due to the indirect effect of macromomycin interacting with the cell membrane. This observation is consistent with the notion that the mammalian cell membrane plays a role in the control of cell metabolism.

Evidence is accumulating that the surface of mammalian cells is a functional structure which controls cell multiplication[6,7,8,9,10]. In this respect, the effect of concanavalin A on transformed cells is of considerable interest[11,12]. Macromomycin inhibits both DNA synthesis and cell multiplication by binding to the cell surface. A similar mode of action is suggested for neocarzinostain because of the related chemical properties of these two drugs, though this assumption has not been tested. Macromomycin might be a useful tool for a study of the function and properties of mammalian cell membranes. Details of these studies and related results will appear in a fuller form.

Table 2. Effect of macromomycin on macromolecular synthesis in YOSHIDA sarcoma cells

Dose	DNA synthesis	RNA synthesis	Protein synthesis
0 (Control)	100 %	100 %	100 %
2 μg/ml	67	100	91
10	57	98	100
50	51	91	100

YOSHIDA sarcoma cells were suspended in EAGLE MEM medium supplemented with 10 % bovine serum (10^{6} cells/ml). DNA, RNA and protein syntheses were measured by determining the incorporation of ^{3}H-thymidine, ^{3}H-uridine and ^{14}C-leucine, respectlvely, into the acid-insoluble fraction. Macromomycin and the radioactive precursors were added at the same time and the incorporation period was 1 hour. The results were expressed as percent of control.

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