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Permeability Control in Animal Cells by Polyenes: a Possibility

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The polyenes amphotericin B and vitamin A enhanced the specific actions of rifampin, fusidic acid, and 1,3-bis(2-chloroethyl)-1-nitrosourea against mouse L cells in tissue culture as measured by macromolecular synthesis and cell survival.

Control of cell permeability seems a distant goal but may be possible in part with a technique we have been using. The technique is based on the use of membrane-active agents at levels that do not detectably harm cell growth or macromolecular formation but perturb the cell membrane to permit penetration of second agents. Detergents and a number of membrane-active antibiotics have been used; this report describes successful trials of the polyenes amphotericin B and vitamin A with mammalian cells in tissue culture. This material has been presented in abstract form (Abstr. 12th Annu. Meeting, Amer. Soc. Cell Biol., St. Louis, Mo., p. 173a, 1972).

Previously, promising trials with yeast have been described in which amphotericin B enhanced the action of second antibiotics by increasing their uptake (3, 4). Considerably higher concentrations of the polyenes are required with the mammalian cells, but Table 1 shows that, as in earlier trials with yeast, either of the polyenes used in combination with one of three second agents had profound effects on protein or ribonucleic acid (RNA) synthesis. The specificity of the second agent was maintained, in that rifampin preferentially affected RNA synthesis, whereas fusidic acid was a more potent inhibitor of protein synthesis. Each of the polyenes or second agents when tested alone had little or no effect on RNA or protein synthesis at concentrations the same as or higher than that used in the combinations.

The effectiveness of the second agent appeared to depend in part on which polyene was being used. For example, rifampin was more potent in combination with amphotericin B than with vitamin A, whereas fusidic acid was more effective with vitamin A. There also appeared to be a difference in sensitivity to the polyenes of the two types of animal cells. Vitamin A was more toxic than was amphotericin B for mouse L cells (Table 1); mouse myeloma cells were more sensitive to the effects of amphotericin B then were L cells (Table 1, Fig. 1). Cell viability was

TABLE 1. *Percentage inhibition of protein and RNA synthesis in mouse L cells exposed to a polyene in combination with a second antibiotic*[a]

Second agent	Polyene			
	Vitamin A, 2 μg/ml		Amphotericin B, 15 μg/ml	
	RNA	Protein	RNA	Protein
None	0	0	0	0
Rifampin				
25 μg	19	0	50	7
50 μg	28	1	58	10
Fusidic acid				
50 μg	15	100	6	48
100 μg	30	100	25	95
BCNU[b]				
7.5 μg	40	—	31	—
15 μg	50	—	61	—

[a] Mouse L cells, grown in spinner cultures with MEM α medium (Flow Laboratories, Rockville, Md.) or MEM, leucine-deficient, plus 5% fetal calf serum, were diluted to between 10^5 and 2×10^5 cells/ml and divided into separate cultures. The separate cultures were incubated with the concentrations of drugs shown and [^{3}H]leucine (0.5 μCi/ml; specific activity, 51 Ci/mmol) or [^{3}H]uridine (0.5 μCi/ml; specific activity, 25 Ci/mmol) for 3 h. Samples of 1 ml were removed in triplicate and centrifuged. The cells were precipitated with 2 ml of 5% trichloroacetic acid; the chilled precipitates were filtered onto glass-fiber filters and counted. None of the second agents caused any inhibition when they were tested with neither polyene present.

[b] 1,3-Bis(2-chloroethyl)-1-nitrosourea.

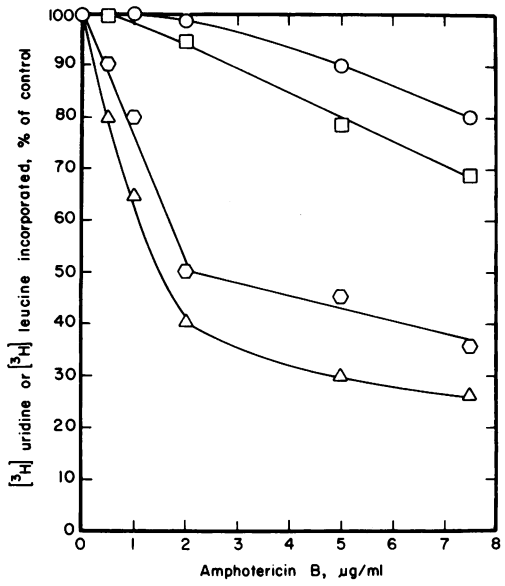

FIG. 1. *Dose response to amphotericin B of [³H]uridine or [³H]leucine incorporation into mouse myeloma cells (MOPC-315) incubated in the presence or absence of rifampin. The incubation and procedure were as described in Table 1. No rifampicin, ○; 29 μg of rifampin/ml, [³H]uridine incorporation, ⬡; 50 μg of rifampin/ml, [³H]uridine incorporation, △; 50 μg of rifampin/ml, [³H]leucine incorporation, □. At 0 concentrations of amphotericin B, only ○ is shown on the figure, but all points were at 100%.*

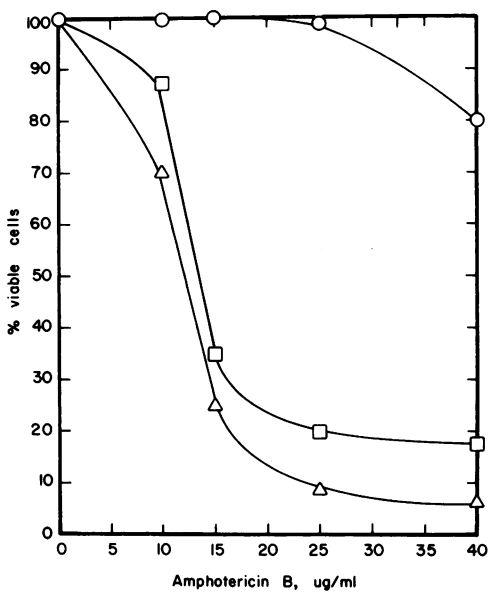

FIG. 2. *Dose response to amphotericin B of the viable-cell count of mouse L cells in the presence or absence of rifampin. Mouse L cells were grown in spinner cultures as described in Table 1. They were diluted to 10^5 to 2 × 10^5 cells/ml and placed in 3.5-cm diameter petri dishes (Falcon) with the concentrations of antibiotics shown. After 18 h, the cells were scraped from the petri dish and counted. Cell viability was determined by trypan blue dye exclusion. No rifampin, ○; 25 μg of rifampin/ml, □; 50 μg of rifampin/ml, △. At 0 concentrations of amphotericin B, only ○ is shown on the figure, but all points were at 100%.*

correlated with the effects on macromolecular synthesis, with consistent results obtained either by cloning the survivors of treatment or by the simpler technique described in Fig. 2 (adapted from Kuwano et al., in press and personal communication).

The results obtained in these experiments correspond closely to the previous findings with yeast (4). In both cases, although the ratios of drugs to cell number were quite critical and had to be carefully standardized, rifampin was effective when used in combination with a polyene. The demonstrated effect of amphotericin B in causing "holes" in the cytoplasmic membrane (2) might be one mechanism by which the potentiation of the second agents is accomplished, though other effects on the enhancement of pinocytosis are also possible.

Independently, Kuwano et al. have obtained evidence that amphotericin B increases the uptake of second drugs. In their studies, both fusidic acid and lentinan, an antitumor polysaccharide extracted from mushrooms, were rendered effective. In another report that may be relevant, Riehm and Biedler (7) used the detergent Tween 80 to increase the uptake of actinomycin D and daunomycin, thereby enhancing the effectiveness of these agents against resistant cells.

The effectiveness of the polyenes on cell membranes is dependent on their binding affinity to sterols. This differs for each polyene. For example, amphotericin B binds much more avidly to ergosterol than to cholesterol (Brajtburg et al., unpublished data) and therefore is an effective antifungal agent (6). Filipin, on the other hand, binds more avidly to cholesterol (5) and is more toxic to animal cells than to fungi. Therefore, sterol content or accessibility in cell membranes may confer relative specificity to the polyenes. The degree of selectivity that can be achieved for permeability control remains to be determined, but recent publications have described the potentiation by vitamin A of antitumor agents (1) and irradiation (8) against in vivo tumors. Those results are consistent with a possible selective effect.

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