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ALTERATION OF CELL PERMEABILITY BARRIERS BY AMPHOTERICIN B–DEOXYCHOLATE (FUNGIZONE) *IN VITRO**

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Abstract— The anti-fungal preparation, Fungizone, a mixture of approximately equal weights of the antibiotic amphotericin B and the ionic detergent sodium deoxycholate, was found to alter significantly the cell membranes of two murine leukemia cell lines. These alterations caused promotion of uptake of actinomycin D and affected two-phase aqueous polymer partitioning. These effects were mainly caused by the deoxycholate component of Fungizone.

Preparations of the anti-fungal agent amphotericin B (Fungizone) are reported to promote the antitumor action of 1,3-bis (2-chloroethyl)-1-nitrosourea (BCNU) [1, 2] and several rifamycin derivatives [3]. Amphotericin B also enhances uptake of intact *Escherichia coli* DNA by HeLa cells, although only at high drug levels [4]. The drug also promotes uptake of 2-deoxyglucose by chick embryo fibroblasts [5] and uptake of both nonelectrolytes and anions by erythrocytes [6]. The drug potentiation studies were apparently not related to the deoxycholate content of Fungizone preparations [2, 3], although this detergent did enhance deoxyglucose uptake in one system [5]. In this study, we have used two sensitive indices of membrane alteration to investigate effects of amphotericin B, deoxycholate and Fungizone on murine leukemia cells.

METHODS

L5178Y cells were grown in Fisher's medium supplemented with 10% horse serum in sealed liter flasks containing 300 ml of the cell suspensions. L1210 cells were grown in nearly full sealed flasks using MEM-Eagle's medium (spinner) containing 10% fetal calf serum. For use, the cells were collected by centrifugation and suspended in fresh growth medium (5×10^6 cells/ml). This medium was buffered at pH 7·3 with *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES) replacing $NaHCO_3$ to permit incubation of high cell density at constant pH. After warming suspensions for 20 min at 37°, the following procedures were carried out:

1. Uptake of [^{3}H]actinomycin D was measured by addition of a stock solution of the drug to 1-ml portions of cell suspensions (final drug concentration = 0·1 μg/ml, 0·05 μCi/ml). At indicated intervals, cells were collected by centrifugation, washed once with cold 0·9% NaCl, then dispersed in an appropriate solvent for liquid scintillation counting. Under standard conditions, the medium contained 85,000 cpm/ml of ^{3}H-labeled drug.

2. To measure effects of surface-active agents on actinomycin D uptake, these were added 10 min before labeled actinomycin D, and the incubation was continued for 10 min before collection of cells.

3. Two-phase aqueous polymer studies were carried out as described by Gersten and Bosmann [7, 8]. After incubations, cells were collected by centrifugation and suspended in 150 mM NaCl. A 0·5-ml portion of this suspension, containing 10^6 cells, was added to a two-phase mixture so that final concentrations were 5% (w/v) Dextran T-500 (Pharmacia), 4% polyethylene glycol (Carbowax 6000, Union Carbide), 50 mM NaCl and 100 mM potassium phosphate at pH 7·0. The total volume was 10 ml. The suspension was gently mixed, and a sample of 0·5 ml was taken and diluted 10-fold with diluent for determination of cell number in a Coulter counter. The phases were then allowed to separate at 4° for 1 hr and the top phase was collected. Another 0·5-ml sample was similarly diluted for counting. The ratio of cells in the top phase to cells in the total system was calculated.

Growth medium was purchased from Grand Island Biological Co.; labeled actinomycin D was provided by Monsanto Chemical Co. (5 mCi/m-mole) and stored in ethanol at −20°. Fungizone was purchased from Grand Island and amphotericin B from Calbiochem Corp.

RESULTS

Uptake of actinomycin D was a function of time and temperature with both L1210 and L5178Y cells (Fig. 1). With 5 mg of cells, a distribution ratio of 10 (intracellular/extracellular drug concentration) = 5000 cpm of radioactivity in the cell pellet. Addition of 200 μg/ml of Fungizone enhanced actinomycin D uptake by both cell lines; after 10 min, drug accumulation was identical in both (dashed line, Fig. 1). When cells were treated for 10 min with 200 μg/ml of Fungizone, then washed free from this agent, the promotion of actinomycin D uptake was not reversed.

Enhancement of actinomycin D uptake could not

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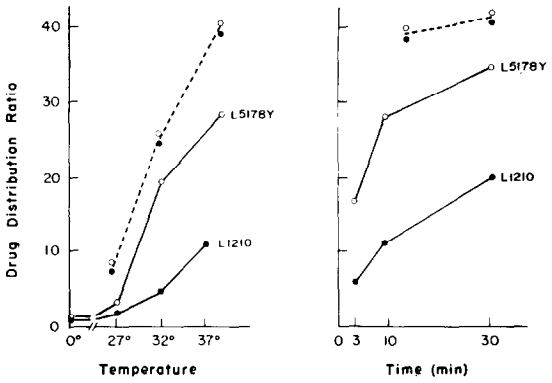

Fig. 1. Uptake of actinomycin D by L1210 (●) and L5178Y (○) cells in the absence (solid lines) and in the presence (dashed lines) of 250 μg/ml of fungizone.

Table 2. Effects of amphotericin B and deoxycholate on partition ratio*

Additions	Partition ratio L1210 cells	L5178Y cells
Controls	0·36	0·50
Deoxycholate, 50 μg/ml (106 μM)	0·35	0·48
Deoxycholate, 100 μg/ml (212 μM)	0·28	0·43
Deoxycholate, 500 μg/ml (1060 μM)	0·10	0·35
Amphotericin B, 100 μg/ml (104 μM)	0·35	0·48
Amphotericin B, 250 μg/ml (260 μM)	0·27	0·43
Fungizone, 100 μg/ml†	0·19	0·40
Fungizone, 250 μg/ml‡	0·11	0·33

* All values are subject to a variation of ±5 per cent. Fungizone is a mixture containing 40% by weight of deoxycholate and 60% amphotericin B.

† 85 μM deoxycholate + 62 μM amphotericin B.

‡ 210 μm deoxycholate + 155 μM amphotericin B.

be obtained by the use of 200–500 μg/ml of amphotericin B, but was found when 100 μg/ml of deoxycholate was employed. The Fungizone-induced promotion of actinomycin D uptake is therefore solely due to the deoxycholate content of the preparation (Table 1).

Two-phase aqueous–polymer partition studies were carried out at pH 7·0 using a standard procedure. Under these conditions, the number of cells partitioning into the top phase was markedly lowered by addition of Fungizone levels which promote actinomycin D uptake (Table 2). Amphotericin B had a slight effect on the partition ratio, but deoxycholate had a more pronounced effect thereon. There is evidence that the amphotericin B–deoxycholate mixture produced an additive lowering of the partition ratio.

DISCUSSION

The anti-fungal agent amphotericin B promotes the antitumor effect of certain drugs via a process apparently not related to the deoxycholate content of the formulation of Fungizone [2, 3]. Deoxycholate is added to enhance solubility of amphotericin B. Medoff *et al.* [2] have suggested that the role of amphotericin might involve stimulation of the immune response in tumor-bearing animals. The present data show that Fungizone, or deoxycholate, but not amphotericin B causes an enhancement of actinomycin D uptake in murine leukemia cells. This was most marked in the L1210 cell line [9], which is relatively insensitive to actinomycin D. Selection for actinomycin D resistance in the drug-sensitive L5178Y cell line was also found to be associated with a barrier to drug uptake [10]. Presumably, an inherent barrier to actinomycin D uptake in the L1210 cell is altered by deoxycholate. The use of another detergent, Tween 80, also promoted actinomycin D uptake by drug-resistant hamster cells [11].

Table 1. Effects of amphotericin B and deoxycholate on actinomycin D uptake by L1210 cells*

Additions	Distribution ratio
Controls	12·0 ± 0·6
Amphotericin B, 250 μg/ml (260 μM)	11·0 ± 0·6
Deoxycholate, 100 μg/ml (210 μM)	14·0 ± 0·7
Deoxycholate, 250 μg/ml (530 μM)	25·3 ± 1·3
Fungizone, 250 μg/ml	21·5 ± 1·0
Fungizone, 100 μg/ml	15·5 ± 0·8

* Fungizone is a mixture containing 40% by weight of deoxycholate and 60% amphotericin B. Therefore, 250 μg/ml of Fungizone = 210 μM deoxycholate + 155 μM amphotericin B, and 100 μg/ml of Fungizone = 85 μM deoxycholate + 62 μM amphotericin B.

Although amphotericin alone failed to stimulate actinomycin D uptake, the drug did cause an alteration in the behavior of cells in the two-phase partition system. The precise nature of the phenomenon being measured is unknown, but it is believed to involve the net charge on and near the cell surface [7, 8, 12, 13].

Amphotericin is known to interact with sterol components of cell surfaces, thereby altering membrane permeability [14]. An extensive depletion of cholesterol was found to be necessary before permeability of the erythrocyte membrane to several nonelectrolytes and anions changed [15]. This finding suggests that relatively high amphotericin levels are necessary to affect permeability of other mammalian cell types, and this suggestion is consistent with data [4] showing that promotion of DNA uptake by HeLa cells was observed only at high (200 μg/ml) levels of amphotericin B. Furthermore, the description offered by Kumar *et al.* [4] does not state whether amphotericin B or Fungizone was employed.

Although we could not detect an effect of amphotericin B on membrane permeability barriers to actinomycin D, effects of this drug on partition behavior do suggest that a cell surface alteration is associated with exposure to amphotericin B. This alteration might be responsible for increased immunogenicity of amphotericin-treated cells, thereby providing an explanation for the synergism between amphotericin B and antitumor agents described previously [1–3].

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REFERENCES

1. G. Medoff, D. Schlessinger and G. S. Kobayashi, *J. natn. Cancer Inst.* **50**, 1047 (1973).
2. G. Medoff, F. Valeriote, R. G. Lynch, D. Schlessinger and G. S. Kobayashi, *Cancer Res.* **34**, 974 (1974).
3. G. Medoff, D. Schlessinger and G. S. Kobayashi, *Cancer Res.* **34**, 823 (1974).
4. B. V. Kumar, G. Medoff, G. Kobayaski and D. Schlessinger, *Nature, Lond.* **250**, 323 (1974).
5. D. Dolberg and M. J. Bissell, *In Vitro* **10**, 26 (1974).
6. B. Deuticke, M. Kim and Chr. Zöllner, *Biochim. biophys. Acta* **318**, 345 (1973).
7. D. M. Gersten and H. B. Bosmann, *Expl Cell Res.* **87**, 73 (1974).
8. D. M. Gersten and H. B. Bosmann, *Expl Cell Res.* **88**, 225 (1974).
9. D. Kessel and I. Wodinsky, *Biochem. Pharmac.* **17**, 161 (1968).
10. D. Kessel and H. B. Bosmann, *Cancer Res.* **30**, 2695 (1970).
11. H. Riehm and J. L. Biedler, *Cancer Res.* **32**, 1195 (1972).
12. D. E. Brooks, G. V. F. Seaman and H. Walter, *Nature, Lond.* **234**, 61 (1971).
13. P. A. Albertson, *Partition of Cell Particles and Macromolecules*, pp. 118–143. Wiley, New York (1960).
14. T. E. Andreoli, *Kidney Int.* **4**, 337 (1973).
15. M. Grunze and B. Deuticke, *Biochim. biophys. Acta* **356**, 125 (1974).