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NOTES

Inhibition of Amphotericin B (Fungizone) Toxicity to Cells by Egg Lecithin-Glycocholic Acid Mixed Micelles

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Mixed micelles prepared from egg lecithin and the sodium salt of glycocholic acid markedly inhibited amphotericin B toxicity to mammalian cells without significantly affecting the antifungal effects of the drug.

Despite its importance in antifungal therapy, amphotericin B (AmB) has serious side effects which are probably linked to its toxic effects on host cells. The clinical formulation of AmB, Fungizone, contains the polyene together with the detergent deoxycholic acid. In vitro studies have shown that in this form AmB is more toxic to fungi than to mammalian cells (1). This selectivity can be further increased by using AmB in complexes with lipids or detergents (2). Here we show that mixed micelles (MixM) composed of egg lecithin and the detergent glycocholic acid further increase the specificity of AmB for fungal cells.

Egg yolk phosphatidylcholine (egg lecithin) and glycocholic acid sodium salt were obtained from Sigma Chemical Co. (St. Louis, Mo.). AmB, in the form of Fungizone, was obtained from E. R. Squibb and Sons (Princeton, N.J.).

MixM were prepared by coprecipitation of egg lecithin and glycocholic acid sodium salt as described by Son and Alkan (9). After the appropriate amount of each lipid (egg lecithin-glycocholate; molar ratio, 1.2) was dissolved in ethanol, the mixture was dried and dissolved in distilled water. Small unilamellar vesicles (SUV) were prepared from egg lecithin as described previously (5).

Human erythrocytes obtained from normal donors, *Candida albicans* B 311, and *Cryptococcus neoformans* 145 A were prepared and assayed as described previously except for minor modifications (3, 5). Different concentrations of MixM or SUV were prepared in a solution of phosphate-buffered saline (pH 7.4) for the erythrocyte assays or in Sabouraud medium for the fungal cell assays. For each assay, a constant concentration of AmB (selected on the basis of preliminary experiments) was used. After 15 min of incubation at room temperature, the AmB-MixM or AmB-SUV mixtures were added to the erythrocytes dispersed in buffered saline or fungal cells dispersed in Sabouraud medium. Final concentrations were 1×10^8 erythrocytes per ml or 2.0×10^7 fungal cells per ml. After 1 h of incubation at 37°C, the cells were harvested by centrifugation and analyzed for retention of K^+ or hemoglobin and, in the case of fungi, viability. Mouse fibroblast L-929 cells were cultured in monolayers in plastic dishes; they were assayed for AmB-induced K^+ leakage and lethality as described previously (3). To measure MixM or SUV effects on the anticellular activities of AmB, 200-μl samples of medium containing constant concentrations of AmB were incubated for 15 min at room temperature with increasing concentrations of MixM or SUV. The mixtures were then added to the cell monolayers overlaid with 800 μl of medium per dish.

Figure 1 shows the effects of MixM on toxicity of AmB for erythrocytes, L cells, and *C. albicans* and *C. neoformans* cells as a function of the concentration of egg lecithin incorporated into the MixM.

MixM effectively inhibited AmB-induced decreases in the retention of K^+ by mammalian cells; MixM were much less potent in inhibiting AmB-induced loss of K^+ by fungal cells (Fig. 1A). MixM were also potent in inhibiting AmB-induced hemolysis of erythrocytes and death of L cells and much less active in inhibiting AmB-induced lethality to fungal cells (Fig. 1B). In both assays, a broad range of concentrations of MixM (from 10 to 100 μg of egg lecithin per ml) effectively inhibited AmB toxicity to mammalian cells without any effect on AmB toxicity to fungal cells.

When used separately, concentrations of glycocholate and egg lecithin SUV up to 400 μg/ml were not toxic to any of the cells assayed. Glycocholate alone did not affect AmB cellular toxicities. Since the inhibitory effect of egg lecithin on AmB action has been demonstrated previously (5), we tentatively ascribe the inhibitory action of MixM to the egg lecithin component.

The molar ratio of the concentration of vehicle required to decrease AmB effects by 50% to the concentration of AmB used can be regarded as an inhibitory index. We compared the inhibitory indices calculated for MixM from the data shown in Fig. 1 with those obtained for egg lecithin SUV from parallel experiments (not shown) and also with those we reported previously for the action of the detergent sucrose laurate (3). Table 1 presents the effects of the vehicles on AmB toxicity to mammalian cells. Inhibitory indices were always greater (2- to 23-fold) when AmB toxicity was measured as a decrease in retention of K^+ than when it was measured as a decrease in hemoglobin or viability. This observation, which was also previously made for the effects of egg lecithin SUV, has been attributed to the differences in AmB concentration required for the different effects (5).

The inhibitory indices calculated for MixM were two- to fourfold lower than those calculated for egg lecithin SUV, indicating that MixM were more efficient in decreasing AmB

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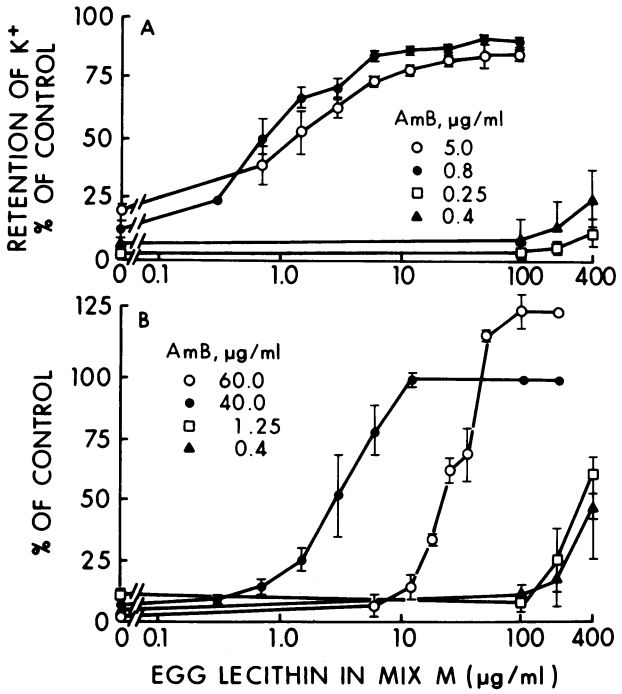

FIG. 1. Inhibition by MixM of AmB-induced decreases in retention of K^+ by cells (A) and retention of hemoglobin by erythrocytes or viability of L cells, *Cryptococcus neoformans*, or *C. albicans* (B). Symbols: ●, erythrocytes; ○, L cells; ▲, *Cryptococcus neoformans*; □, *C. albicans*. Concentrations of AmB used are shown. Data are means ± SE from three experiments.

toxicity. According to the model of in vitro inhibition of AmB toxicity by a vehicle that we have discussed elsewhere (2), the decrease in toxicity is caused by AmB binding to the vehicle. Thus, the fact that the inhibitory potency of MixM is greater than that of SUV suggests that AmB binds more to the egg lecithin component in MixM than to the egg lecithin in SUV, or alternatively, that in addition to binding to egg lecithin in MixM, the AmB binds also to the glycocholate component of the MixM. The inhibitory indices calculated for the effects of sucrose laurate on AmB-induced decreases in retention of K^+ by erythrocytes and L cells were comparable to those obtained for egg lecithin SUV. The inhibitory indices calculated for the effects of sucrose laurate on hemolysis of erythrocytes and viability of L cells cannot be compared with those for egg lecithin SUV or MixM, because different concentrations of AmB were used in the former experiments (3).

The selectivity of the inhibitory effect of the MixM on AmB cellular toxicity adds this vehicle to the list of other compounds or formulations that decrease AmB toxicity to mammalian cells without markedly decreasing the effects of AmB on fungal cells (3–5, 7). Some of these other vehicles have already been shown to decrease AmB toxicity to animals (4, 10) or even patients (6) without impairing the antifungal activity of AmB. It is probable that the in vitro selectivity of MixM may also be reproduced in animals. If this assumption is confirmed, MixM will be excellent candidates for use as vehicles in AmB therapy because they have the following desirable properties: they are nontoxic to cells, easy to prepare, and very stable (11), and they are composed

TABLE 1. Indices calculated for inhibition of AmB toxicity to cells by vehicle

Vehicle	Inhibitory index[a]			
	Erythrocytes		L cells	
	K^+	Hemoglobin	K^+	Viability
Egg lecithin in MixM[b]	2.1	0.1	1.2	0.55
Egg lecithin SUV[b]	9.0	0.4	6.0	1.16
Sucrose laurate[c]	10.0	1.6	5.6	0.24

[a] Defined as the molar ratio of the concentration of vehicle required to decrease AmB effects by 50% to the concentration of AmB used.
[b] Cells were treated with AmB at concentrations specified for each assay in Fig. 1. In the absence of a vehicle, cells retained 10 to 20% of measured indices; in its presence, retention was 60 to 70%.
[c] Data from reference 3.

of two biological components (egg lecithin and bile salt) and have already been used in clinical trials as a vehicle for delivery of other drugs (8).

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