MINIREVIEW

Amphotericin B: Delivery Systems†

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INTRODUCTION

Amphotericin B (AmB) is insoluble in aqueous solution and before it can be used clinically as an antifungal agent to treat systemic mycosis, a vehicle (carrier) has to be added to form a dispersion. The commercial preparation of AmB, Fungizone, is a mixture of AmB, a detergent deoxycholate, and a buffer. When suspended in a glucose solution, Fungizone forms a colloidal dispersion suitable for intravenous injection. AmB can also be obtained as a dispersion by addition of a concentrated AmB solution in organic solvents to water; this preparation has been used in several in vitro studies on the cellular and molecular effects of AmB. Because the clinical utility of AmB is limited by its toxicity to host cells, an important question is how to best direct it specifically to the fungus or at least to the site of infection or, alternatively, how to keep it away from the host cells. One strategy is to use a vehicle other than deoxycholate. In this minireview, we review some of the relevant studies addressing this issue.

LIPOSOMES

The term liposome is used here interchangeably with the term lipid vesicles and refers to phospholipid bilayers of one or more closed concentric structures. The first attempt to use antifungal agents in combination with liposomes was guided by the previous findings of an enhancement of the antileishmanial activity of antimony compounds by liposomes (27). The rationale for the work on AmB was that because both the parasites and liposomes were taken up by phagocytic cells, the AmB entrapped in the liposomes might be brought into close proximity to the parasite inside the host macrophages. Results of this work demonstrated that preparations of liposomal AmB were less toxic to the host and had higher therapeutic capability than dispersions of free AmB.

In subsequent studies, liposomes have been used as vehicles for AmB in treatment of murine histoplasmosis (31), cryptococcosis (8), and candidiasis (24). In all of these experimental systems, as well as in additional studies (for references, see references 20 and 32), liposomal AmB was shown to be as potent as the free drug against infecting fungi, whereas its toxicity to the host was decreased. The lower level of toxicity permitted larger doses to be given, and consequently the liposomal formulations were more effective than free AmB against fungal infections in the animal models used.

Basically the same pattern of response was observed in three pilot clinical studies on the use of liposomal AmB in cancer patients with fungal infections (21, 22, 29). Patients tolerated liposomal AmB much better than AmB in the form of Fungizone. Increased doses could be administered, and a significant clinical improvement was observed in some patients. Although these studies are difficult to evaluate because the patients had advanced neoplastic disease and multiple concurrent therapies, the results justify further investigation. As Sculier et al. (29) emphasized, the type of liposomal vehicle (chemical composition, charge, structure, and mode of preparation) can modify the physicochemical, biological, and pharmacological properties of liposomes and thus of the carried drug. Because the mechanism(s) by which liposomes improve the therapeutic index of AmB is not yet completely elucidated, the effects of all these variables are not predictable and require further study.

The initial idea that liposomes act specifically by directing AmB to the site of infection gained support from observations that the delivery of AmB as a liposomal preparation markedly altered the disposition of the drug. However, no association between these alterations and decreased toxicity was found (23, 24). Moreover, in other studies no significant differences in organ distribution between the liposomal and free AmB have been found (30).

A general correlation between results obtained in vitro and in vivo was shown by Szoka et al. (30), who demonstrated that liposomal AmB was less cytotoxic to cultured murine cells and less toxic to mice than free AmB. A partial understanding of events involved in liposome effects in vivo comes from investigations on the cellular and molecular level which demonstrated that AmB administration in liposomes displays reduced toxicity to human cells (erythrocytes) while maintaining activity against Candida albicans fungal cells (25).

The biochemical basis of the enhanced therapeutic index of liposomal AmB has been further explored by experiments that show that free AmB but not liposomal AmB induced cation release from erythrocytes and from liposomes composed of erythrocyte membrane lipids (15). The parallel responses of erythrocytes and liposomes composed of lipids from erythrocytes indicate that the lipids in the erythrocyte membrane were responsible for the difference in sensitivity to free and liposomal AmB. It was further shown that the ability of liposomal AmB to induce cation release and to cause toxicity to erythrocytes could be modulated by changing the lipid composition of the liposome vehicle. These observations were interpreted as indicating that the selectivity of liposomal AmB preparations against the various targets resulted from selective transfer of AmB from "donor" liposomal membranes to "target" cellular membranes (15). This transfer from vehicle to cell occurs by the diffusion of free AmB through an aqueous phase, similar to the previ-

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ously documented transfer of AmB between liposomes (1). The toxicity to cells is thus dependent on the attainment of a sufficient level of AmB in the aqueous phase. The decisive role the concentration of free AmB in the aqueous phase has for the induction of toxicity to cells was clearly demonstrated in studies of the anticalcicular action of mixtures obtained by incubation of AmB with small unilamellar vesicles prepared from saturated phospholipids. These experiments show that the activity of the different preparations of small unilamellar vesicle-AmB against erythrocytes, *C. albicans*, and *Candida neoformans* could be attributed to the activity of the AmB remaining unbound to the small unilamellar vesicles (16, 17; S. Jullien, J. Brajtburg, and J. Bolard, Biochim. Biophys. Acta, in press). Because AmB is more potent against fungal than mammalian cells, the concentration of this residual AmB may in some experimental conditions be sufficient for induction of effects on fungal cells but not sufficient to affect erythrocytes.

**LIPIDS AND DETERGENTS**

If liposome vehicles act by binding AmB and affecting its availability to cells, then the closed structure of liposomes is not required for the increased selectivity in AmB activity seen in vitro and in vivo. In agreement with this notion, it was found that the AmB complexed to Intralipid, a commercially available lipid formulation, behaved in a fashion similar to that of AmB complexed to liposomes (18). The AmB-Intralipid formulation was less toxic to mammalian cells in vitro and had lower acute toxicity than the AmB-deoxycholate formulation after rats and mice were treated. The efficacies of the two preparations against *C. albicans* in vitro were comparable (18). A decrease in AmB toxicity to rats and an attenuation of AmB toxicity to mammalian cells but not to fungal cells were also observed with lipid-stabilized AmB aggregates (14). These lipids had a ribbonlike structure rather than the closed circular form expected of liposomes. These authors observed that the lipids used in these experiments caused changes in the electronic absorption spectrum of AmB and concluded that the decrease in AmB toxicity was not the result of mechanical entrapment in the vehicle but rather the result of lipid-induced changes in the aggregation state of AmB (14). The increase in AmB specificity, as measured by relative toxicity to erythrocytes and fungal cells, was also seen when cholesterol or lipoprotein was present in the reaction mixtures (2). The addition of cholesterol to the dispersion of AmB inhibited its toxicity to erythrocytes more than to fungal cells; the addition of lipoproteins still inhibited AmB toxicity to erythrocytes without affecting its antifungal toxicity. The extent of inhibition depended on the type of lipoprotein used and correlated with the affinity of AmB binding to cholesterol in the lipoproteins. The affinity of AmB binding, as measured by circular dichroism spectroscopy, was greater to low-density lipoprotein than to high-density lipoprotein; the extent of inhibition for equal cholesterol concentrations was also greater for low-density than for high-density lipoprotein. Recently, using an immunosuppressed rabbit model of invasive aspergillosis, Patterson et al. (27a) showed that preparations of cholesterol sulfate complexed to AmB were more effective than equivalent doses of AmB-deoxycholate, presumably because of equivalent antifungal actions and a fourfold decrease in toxicity of the former.

On the basis of these results, we hypothesized that substances other than lipids that bind AmB might also be useful as vehicles for AmB delivery. We investigated the effects of several different detergents on the properties of AmB. Deoxycholate, a detergent used for AmB solubilization (Fungizone), has a small effect on the spectrum of AmB and on its anticalcicular action against fungi and animal cells. A greater effect on the spectrum of AmB dispersed in aqueous solution in the UV-visible region was caused by addition of the detergent Triton X-100. In the presence of selected concentrations of Triton X-100, AmB formed complexes with ergosterol but not with cholesterol (11). It was found that a mild detergent, the lauryl ester of sucrose, formed complexes with AmB under condition which allowed the formation of AmB-ergosterol complexes but not AmB-cholesterol complexes. Furthermore, this ester inhibited AmB toxicity to mammalian cells about 1,000-fold more than toxicity to fungal cells (4, 10). Experiments with noninfected mice have demonstrated that AmB administered together with the lauryl ester of sucrose was much less toxic than when it was administered as Fungizone (manuscript in preparation).

**MODEL**

The results of all these investigations can be accommodated in one model of the action of vehicles on the effects of AmB. The basis for this model (formulated in Jullien et al., in press) includes studies on the molecular level (1) and on the cellular level (7, 14).

AmB may bind to several compounds or formulations which can be used as vehicles. The vehicles that have been tested are liposomes which are closed phospholipid structures (15-17; Jullien et al., in press); open lipid structures (14); lipid formulations such as Intralipid (18); and cholesterol in dispersion or in lipoprotein (2) and detergents, Triton X-100 (11) and lauryl ester of sucrose (4, 10). When the AmB is exposed to the vehicle, an AmB-vehicle complex is formed; this complex exists in equilibrium with AmB remaining free. The extent of complex formation depends on the affinity of AmB binding to the vehicle and on the initial concentration of the vehicle in the reaction mixture. It also depends on the initial concentration of AmB, which determines its aggregation state (9). Because the AmB concentration in the aqueous phase is determined by partitioning from the vehicle preparation, the higher the affinity of the donor, the lower the equilibrium concentration of AmB in the aqueous phase. Preparations containing AmB complexed to the vehicle and free AmB can be treated as AmB donors. When the target or “acceptor” (sterols in dispersion or in artificial or biological membranes) is exposed to the donor, AmB in the donor preparation may transfer through the aqueous phase to the target.

The affinity of AmB to ergosterol or ergosterol-containing artificial and biological membranes is higher than to cholesterol and cholesterol-containing membranes (see reference 5). In addition, the induction of toxicity to mammalian cells requires higher AmB concentrations than does induction of toxicity to fungal cells. The low equilbrium concentration of AmB in the aqueous phase may still be sufficient for induction of toxicity to fungal cells but too low for induction of toxicity to mammalian cells. As stressed by Cybulsky et al. (6), the improvement of selective toxicity is always associated with a decrease of polyeone effectiveness toward cholesterol-containing membranes.

The extrapolation of this model to animal studies should be made with caution. A correlation observed in several studies between the decreased toxicity to mammalian cells in vitro and decreased toxicity to animals should not be taken as a general rule. The toxicity in vivo of the vehicle or of the
TABLE 1. Effects of lipoproteins and lipid vesicles on activity of polypeptide antibiotics against erythrocytes and C. albicans

| Antibiotic     | Effect in vehicle | Lipoprotein | Vesicles |
|----------------|-------------------|-------------|----------|
| Heptaene       |                   |             |          |
| Candidin       | d                  | d           |          |
| Candidin       | d                  | d           |          |
| Meparinic A    | d                  | d           |          |
| Nystatin*      | d                  | d           |          |
| Nonheptaene    |                   |             |          |
| Filipin        | n                  | n           |          |
| Etruscomycin   | n                  | n           |          |
| Fungichromin   | n                  | n           |          |
| Natamycin      | n                  | n           |          |

* Effects of lipoproteins (3) and lipid vesicles (26) are designated as ‘‘d’’ (differentiating effect, vehicle protected erythrocytes but not fungi) or ‘‘n’’ (nondifferentiating effect, lipoproteins protected both types of cells, lipid vesicles did not protect any cell type).

** Nystatin, a tetaene and a diene, is classified together with the heptaenes.

The vehicle-AmB complex can be assayed only by in vivo experiments. For example, lipoprotein inhibited in vitro AmB toxicity to erythrocytes (2) but potentiated its toxicity to rabbits (19).

POLYENE ANTIBIOTICS OTHER THAN AmB

The concept that the greater avidity of AmB binding to ergosterol than to cholesterol is determined by structural characteristics of this polypeptide and sterol molecules and is only potentiated but not imposed by complexing AmB to vehicle is supported by experiments with other polypeptides. Table 1 shows that lipoproteins (3) and lipid vesicles (26) inhibited toxicity of the heptaene polypeptides and nystatin to mammalian cells but not to fungal cells. These polypeptides bind more avidly to ergosterol than to cholesterol. In contrast, lipoproteins and lipid vesicles equally affected the toxicity of nonheptaene antibiotics to mammalian and fungal cells. These polypeptides do not preferentially bind to ergosterol. In addition, liposomical forms of nonheptaenes were as toxic to mice as the same polypeptides administered without liposomes, whereas liposomical forms of nystatin and candidin (heptaene) were less toxic than when the agents were administered without the liposome carrier (26).

CONCLUDING REMARKS

Improving the specificities and the therapeutic indices of AmB and other heptaenes by combining them with vehicles is an exciting and potentially useful area of research. It will likely result in better AmB preparations than Fungizone. It is probable that these vehicles function by forming complexes of AmB which are in equilibrium with free AmB. The latter is delivered to cells which bind AmB with a higher affinity than they bind the vehicle. The implication of this model is that vehicles other than liposomes may improve the therapeutic effects of AmB. Several of these are under active investigation. The early clinical results of treatment with liposomal preparations of AmB are very interesting and should be expanded. The development of proliposomes (28) can help to overcome the difficulties connected with the standardization and stabilization of liposome preparations. Recent efforts at targeting AmB to antigenic sites on fungi by using liposomal AmB formulations with attached specific antibodies might further increase the therapeutic efficacy of AmB (12, 13).

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