SOLUBILIZATION OF AMPHOTERICIN B WITH γ-CYCLODEXTRIN

Sir:

Amphotericin B (AMB) is a non-aromatic heptaene polyene macrolide antibiotic, produced by *Streptomyces nodosus*. Its antimycotic effects are related to its ability to form complexes with sterols, particularly with ergosterol, a component of the fungal cell membrane. Amphotericin B is the only member of the polyene antibiotic group which is used for treatment of the "deep seated" systemic fungal infections. For parenteral administration amphotericin B is solubilized with sodium deoxycholate (Fungizone Intravenous). Fungizone Intravenous forms a colloidal dispersion when reconstituted with sterile water[1]. The infusion solution is prepared by further dilution with 5% Dextrose Injection USP. Despite its side effects amphotericin B remains the most important drug for managing the majority of systemic fungal infections.

On account of its considerable toxicity and low water solubility, the utilization of amphotericin B in therapy is limited. Many attempts have been taken to overcome these problems. Preparation of derivatives of AMB (including methyl ester[2], *N*-acetyl[3], or *N*-D-ornithyl[4]) with increased solubility, but with decreased antifungal potency has been reported. It is possible that the structure of AMB may be optimal and that any modification decreases its activity.

An alternative is solubilization with detergents or complexing agents. AMB solubilized with detergent like substances (*e.g.* sodium lauryl sulfate or sodium deoxycholate[5]) do not form true solutions, only colloidal dispersions in aqueous medium. The solubility of the drug may be enhanced by coprecipitation with polyvinylpyrrolidone[6]. Borate complexes of AMB are about 10-fold more soluble in physiological saline than is AMB, however a monomeric solution is not obtained, only a heterodispersed colloidal solution[7].

We have observed that AMB forms a soluble inclusion complex with γ-cyclodextrin (γ-CD) and that the solubility of AMB can be enhanced up to 200-fold.

Cyclodextrins are widely recommended in the pharmaceutical and food industry, utilizing their complex forming ability. Numerous specific effects can be achieved with inclusion complex formation, *e.g.* materials which are sensitive to oxygen, light or heat, can be protected against oxidation or decomposition, liquid substances can be transformed into crystalline forms, solubility of poorly water-soluble substances can be increased while enhancing absorption and bioavailability, undesired side effects of drugs can be avoided, *etc.*[8~10]. γ-CD would appear to be a potentially useful new injectable carrier, because of its favorable solubility and toxicological properties[11].

Preliminary experiments, as it was foreseen, demonstrated no significant interactions between α- or β-CD and AMB. For similar molecules the γ-CD seems to be the desirable "host-molecule".

Excess amounts of amphotericin B were added to the aqueous solutions containing various concentrations of γ-cyclodextrin. Each suspension was stirred at 37°C for three hours and filtered. The filtrate was diluted with 50% ethanol, and analyzed for amphotericin B content by spectrophotometry using absorption at 407±1 nm. Results in Table 1 show that maximum solubility was achieved after three hours in 10% (W/V) γ-CD solution.

Solid water soluble AMB-γ-CD complex was prepared by freeze-drying the γ-cyclodextrin-AMB solution[12]. The resulting yellow amorphous powder was readily soluble in water, its AMB content between 0.6~0.7%. 100 mg complex can be dissolved easily in 0.6 ml of water and in 0.9% (W/V) NaCl solution, giving a dark yellow clear solution, containing approximately 1 mg/ml dissolved AMB as determined by spectrophotometry.

The antifungal activity of the complex was tested against *Saccharomyces cerevisiae* by agar-diffusion assay method according to British Pharmacopoeia 1980, and was found to be identical with that of the parent compound.

AMB-γ-CD complex solution can be diluted to an unlimited degree with water or isotonic saline without precipitation. The fact that sodium chloride did not result in precipitation of AMB in presence of γ-CD indicates that a true solution is present, because the addition of electrolites to aggregated colloidal dispersed AMB systems results in immediate precipitation. The AMB content of aqueous solutions of the

Table 1. Solubility of amphotericin B in aqueous γ-CD solution.

Concentration of γ-CD (mg/ml)	Concentration of AMB (mg/ml)
0	0.003
20	0.12
40	0.25
50	0.30
60	0.36
80	0.51
100	0.65
120	0.65

γ-cyclodextrin complex stored at ambient temperature and not protected from light showed no spectral change after six days of storage.

In DMSO-ethanol solution AMB shows a very characteristic UV-absorption spectrum having three sharp intense maxima at 407, 382 and 363 nm, which is characteristic for the monomer. The presence of a peak at 325 nm and reduction of the peaks between 350~420 nm is characteristic for the aggregation of monomeric AMB molecules.

According to UV-visible and circular dichroism spectra molecules of AMB solubilized by detergent type substances are highly aggregated in aqueous media. When increasing amounts of γ-CD are added to such a colloidal system its spectral properties progressively approximate those characteristic of true molecular-dispersed AMB solutions. Apparently γ-CD reverses the aggregation of micelle-like associates of AMB. Ethanol of 50% concentration results in a similar effect[13].

The spectrum of AMB in water in the presence of about 2×10^{-3} M γ-cyclodextrin is identical in intensity and shape with that in DMSO-ethanol. Amphotericin B-γ-cyclodextrin complex presents potential advantages for use by parenteral administration because of enhanced solubility and the formation of a true aqueous solution. An undesirable property of AMB solubilized with bile salt is that it can be precipitated during long-term infusion, resulting in severe side effects[14] to the patient. This precipitation can be prevented by adding γ-cyclodextrin to the solution. The γ-cyclodextrin complex also offers the possibility of examining the role of bile salt in the toxicity of Fungizone. Some question whether the apparent increase in toxicity is due to the bile salt, or to the increased availability of solubilized AMB. Animal toxicity tests have shown, that the more soluble and finely dispersed solution of AMB methyl ester was less toxic than the parent compound[15], indicating that an increase in availability *per se* does not result in an increase in toxicity.

An important advantage of the AMB-γ-CD complex is the simplicity of preparation, making it suitable for preparing test solutions to study the interaction of lipid membranes to elucidate the underlying mechanism responsible for the antifungal activity of polyenes.

Amphotericin B type I. reference powder was generously supplied by E.R. Squibb and Sons Inc. Princeton, N.J. USA. Amphotericin B reagent grade used in experiments was bought from Sigma (St. Louis, MO. USA.), γ-cyclodextrin is a product of CHINOIN, Budapest, its minimum purity was 99%.

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