A BIOLOGICALLY ACTIVE BORATE DERIVATIVE OF AMPHOTERICIN B SOLUBLE IN SALINE SOLUTION

Sir:

The poor solubility and high degree of aggregation of amphotericin B and other polyene macrolides has made it difficult to study the interaction of these antibiotics with lipid membranes and to elucidate the underlying mechanisms responsible for their antifungal activity, their effect of lowering of serum cholesterol levels, and their toxic effects. We, therefore, undertook the preparation of a biologically active derivative of amphotericin B with sufficiently high solubility and stability under physiological conditions of pH and salt concentration to make such studies possible.

In previously reported derivatives of amphotericin B increased water-solubility was obtained by elimination of amphotericity or by prevention of stacking by micellar solubilizing agents. The first approach includes N-acylation which produces water-soluble derivatives but greatly reduces antifungal activity;[1] esterification of the carboxyl group which results in a water-soluble methyl ester hydrochloride with unimpaired activity;[2] and reaction of the free amino group of the antibiotic with a carbohydrate, giving water-soluble N-glycosyl derivatives.[3] Examples of the second approach include dispersion in sodium desoxycholate as in the case of Fungizone^{R}, or adsorption on poly(vinylpyrrolidone).[4] We here report the preparation of a borate derivative of amphotericin B which is soluble in water and saline solutions and retains most of the biological activity.

A mixture of methanol and DMSO (dimethyl sulfoxide) proved suitable as a common solvent for amphotericin B and sodium tetraborate decahydrate (borax). The solubility of the latter is 2.7×10^{-3} M in pure DMSO and increases almost linearly with increasing amounts of methanol to 115.8×10^{-3} M in pure methanol (at 25°C). The parent compound (designated AB) was dissolved in a 7:3 (v/v) mixture of DMSO and methanol which was saturated with $Na_2B_4O_7\cdot10H_2O$ and contained a small excess of solid borax. The solubility of borax in this solvent mixture was found to be 42.4×10^{-3} M. The dissolution of AB in the saturated borax solution was aided by a vortex mixer or ultrasonic cleaning bath. The resulting solution was filtered to remove excess borax and undissolved AB. The filtrate was added dropwise to a ten-fold volume of acetone in which the amphotericin B-borate complex (designated AB-borate) precipitated. The precipitate was collected on a fritted glass filter funnel and vacuum-dried at 40°C. It was found that borate had to be present in excess in order to obtain virtually complete conversion of AB to the borate complex. Since borax is practically insoluble in acetone, the precipitate formed in the latter consisted of the stoichiometric AB-borate complex and excess borax, amounting to 10~15% of the precipitate. The latter could be removed by dialysis or ultrafiltration, since, as found subsequently, AB-borate has a high molecular weight.

To determine solubilities, excess amounts of AB-borate and AB were suspended in water and isotonic (0.9%) NaCl solutions which were adjusted to different pH values by addition of very dilute HCl or NaOH. The suspensions were allowed to equilibrate for 12 hours on a slow-moving wrist-shaker in small vials with conical bottoms. Subsequently, these were centrifuged at 4,500 r.p.m., and the supernatant solutions analyzed for amphotericin B by absorbance at 408 nm.

Results are given in Table 1 which shows that AB-borate has a minimum solubility at pH 5~6 of 0.54 mg/ml in water, and of 0.020 mg/ml in isotonic saline. This compares with minimum solubilities of the AB parent compound, at about pH 5, of 0.003 mg/ml in water and 0.002 mg/ml in isotonic saline. All solubilities increase sharply in acid or alkaline solutions.

The AB-borate compound was characterized by examining its composition, molecular weight, and absorption spectrum. Analysis for borate by a fluorescence method and for polyene by spectrophotometry gave a molar ratio of boron to polyene of 2.65 in neutral solutions (pH 6.3). The molecular weight was determined by differential ultrafiltration through a series of filters having molecular weight cut-offs ranging from 500 to 200,000 daltons. At pH 7 the AB-borate compound had a molecular weight of the order of 100,000 daltons. Since the monomer molecular weight is about 1,000, this corresponds to a polymer of 100 units. The absorption spectra of a series of solutions of increasing concentration showed a progressive change from a well-resolved

Table 1. Solubilities of AB and AB-borate in mg/ml

pH	AB (no salt)	AB (0.9% NaCl)	pH	AB-borate (no salt)	AB-borate (0.9% NaCl)
1.47	0.129	0.013	2.50	3.90	0.125
4.90	0.003	0.002	5.1	0.54	0.020
5.75	0.003	0.002	5.70	0.54	0.020
7.46	0.073	0.038	6.20	0.55	0.110
8.94	1.25	0.846	7.43	4.70	4.70
10.67	5.28	3.02	8.70	130	120

spectrum, characteristic of AB in methanol, to an unresolved spectrum characteristic of colloidal dispersions of AB in water. For AB-borate, the concentration at which the colloidal-type spectrum appeared was about 200 times greater than that of AB.

From these data it appears that AB-borate exists as a polymer which, at least in dilute solutions, contains fully solvated polyene groups rather than clusters or stacks of such groups as shown by the spectrum. It may therefore consist of long chains of alternating polyene and borate groups of the type $-(AB-B)_n-$ and $-(AB-B_4)_n-$ where B is orthoborate, containing one boron, and B_4 is tetraborate, containing four boron atoms. The observed ratio of boron to polyene indicates that both types of borate groups are present. The formation of such chains is made possible by the bifunctional character of borate and tetraborate towards diol compounds.[5] The AB molecule in turn is bifunctional towards borate since it has 1,2- and 1,3- diol groups on the macrolide ring, and vicinal amino- and hydroxyl-groups on the mycosamine moiety.

The AB-borate compound was tested for antifungal activity, along with the parent compound, against *Saccharomyces cerevisiae* by a turbidimetric assay as described by BONNER, *et al.*[6] The test systems were incubated at pH 5.5. The activity of AB-borate was found to be 80% of that of the parent compound. Since the borate complex is stable in this pH range, as shown by the borate analysis, it may be concluded that the antifungal activity was due to the complex itself, rather than to free amphotericin B formed by the decomposition of AB-borate in the culture medium. The partial loss in activity may result from complexation of the amino group with borate, since acylation of this group also reduced the activity.[1]

In view of its biological and physical properties, this borate derivative appears to be suitable as a test compound for investigating the interactions of amphotericin B with lipid membranes and their constituents.

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