STABILITY STUDIES WITH AMPHOTERICIN B AND AMPHOTERICIN B METHYL ESTER

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In solid form, amphotericin B and amphotericin B methyl ester free base exhibit similar stability. Acid salts of the methyl ester derivative stored under identical conditions are less stable. In solution, amphotericin B is generally more stable than its methyl ester salts. However, when pH is adjusted to 6.0 and storage temperature held at 5°C the methyl ester salts reflect the stability exhibited by the parent compound, amphotericin B.

Amphotericin B (AB), a polyene macrolide antibiotic, is of considerable importance in the treatment of systemic mycosal infections, despite its high toxicity[1]. The recent introduction of the methyl ester of amphotericin B (AME), which shows increased aqueous solubility and decreased toxicity from the parent compound, yet displays a good retention of antifungal activity, holds promise for a better therapeutic approach to fungal infections[2~5].

The polyene macrolide antibiotics, as a group, exhibit poor stability when exposed to heat, ultra-violet irradiation and extremes of pH[6,7]. Inactivation of these antibiotics from aerial oxidation through the formation of epoxides has also been noted[8].

We have found that stability is an important consideration in the determination of the clinical utility of AME, due to its highly dispersed nature in aqueous media and increased accessibility to deleterious influences. In this report we are concerned with the problem of relative stabilities of amphotericin B, its methyl ester and methyl ester salts in regard to the effect of both heat and pH on bulk and solution preparations.

Materials and Methods

Antibiotics: Amphotericin B was evaluated as Fungizone®, the commercial formulation employing sodium desoxycholate as a solubilizing agent. Amphotericin B methyl ester and its hydrochloride and ascorbate salts were prepared in our laboratories according to methods previously described[2,3].

Bioassays: A turbidometric assay using *Saccharomyces cerevisiae* (ATCC 9763) and SABOURAUD's medium (4% dextrose, 1% Neopeptone) as the test system was employed. Antibiotics were dissolved in either dimethyl sulfoxide or sterile distilled water for assay and suitable diluent controls were run. A logarithmically growing culture was adjusted to 80% light transmission at 550 nm as measured by a Coleman Model 44 Spectrophotometer. This stage was diluted 1:100 with fresh medium and dispensed in 5-ml volumes to which various concentrations of the antibiotics were added. After incubation for 24 hours at 28°C the test was read at 550 nm using the above spectrophotometer. Throughout the test, 3~5 concentrations were run for each preparation with 4 replicas being made per concentration. Standard curves for each starting preparation were constructed using 6~8 concentrations (4 replicas

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per concentration) of the sample in question. In both situations results were averaged. Bioactivity of the preparations stored under various conditions throughout the test was determined by comparison to the respective standard curve.

Storage Conditions: One dram vials (45×13 mm) were filled to the half-way point with the preparation to be tested and stoppered in the presence of air. Vials were then covered with aluminum foil to protect the contents from light.

Bulk Stability: Four bulk preparations were evaluated: AB, AME free base, AME·HCl (pH 4.0) and AME·HCl (pH 6.5). These preparations were stored in a +60°C oven for a period of up to 21 days during which time samples were taken and subjected to bioassay.

Solution Stability: Again four preparations were evaluated: AB, AME·HCl (pH 4.5), AME·HCl (pH 6.0) and AME·ascorbate (pH 6.0). The preparations were each made up to concentration of 0.5% with sterile distilled water. They were then stored at either 5°C or 28°C for a period of 13 days during which time samples were taken and bioassays performed.

Results

As shown in Fig. 1, the bulk preparations of AB and AME free base stored at +60°C exhibit similar stabilities over a period of 21 days. The hydrochloride salts of AME exhibit inferior stabilities when stored under the same conditions for a shorter period of time. However, this increased rate of deterioration for the AME·HCl salts seems to be a pH dependent process in that the sample at pH 6.5 shows a slower loss of activity than the sample at pH 4.0.

The solution stabilities of AB and the AME salts stored at 5°C and 28°C are shown in Figs. 2 and 3 respectively. AB in aqueous solution demonstrates good stability at both 5°C and 28°C. The rates of degradation for AME·HCl (pH 4.5) in aqueous solution were greatly increased from that observed for AB. However, when the pH of the AME·HCl preparation was raised to 6.0, the rates of deterioration at 5°C and 28°C were significantly decreased. At 5°C, AME·HCl (pH 6.0) had comparable stability to AB stored under the same conditions. The stability of AME·HCl (pH 6.0) stored at 28°C, while decreased from that of the sample stored at 5°C was generally better than that of the pH 4.5 preparation.

The ascorbate salt of AME (pH 6.0) in aqueous solution exhibited good stability at 5°C.

Fig. 1. Relative stabilities of AB, AME (free base), AME·HCl (pH 6.5) and AME·HCl (pH 4.0) stored in solid form at +60°C.

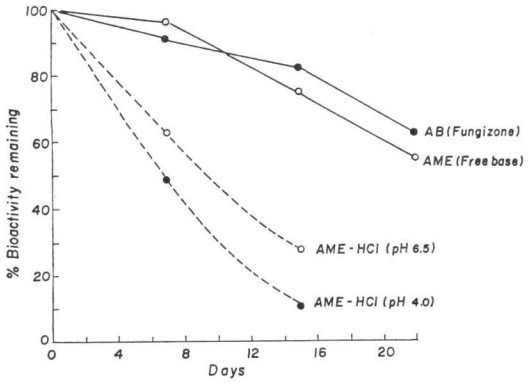
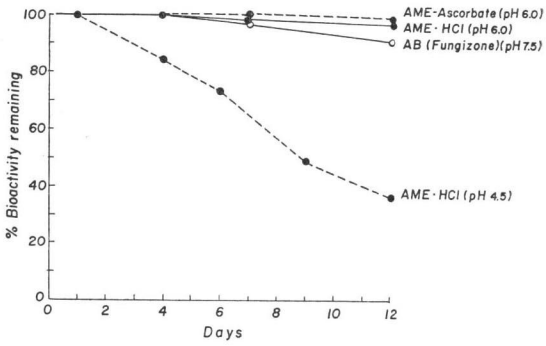
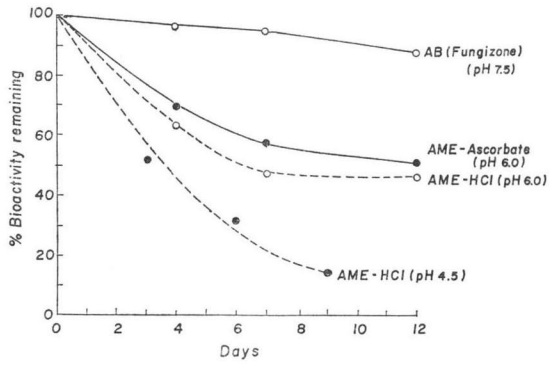


Fig. 2. The effect of pH on the solution stability of AME·ascorbate (pH 6.0), AME·HCl (pH 6.0), AB (pH 7.5) and AME·HCl (pH 4.5) stored at 5°C
Concentrations: 0.5% in distilled water



At 28°C the rate of degradation of this salt was increased as would be expected. While the stability of the ascorbate salt may seem to be greater than that observed for the hydrochloride salt when both were stored at 28°C, the differences in bioactivity were generally not significant. Likewise, at 5°C there were no significant differences in the stabilities of the hydrochloride and ascorbate salts.

Fig. 3. The effect of pH on the solution stability of AB (pH 7.5), AME·ascorbate (pH 6.0), AME·HCl (pH 6.0) and AME·HCl (pH 4.5) stored at 28°C
Concentrations: 0.5% in distilled water



Discussion

The constrained nature of the macrolactone ring due to an extended unsaturated chromophore, and the presence of a variety of functional groups confer on AB a sensitivity to diverse chemical and physical attack. Products resulting, with their altered chemical and physical properties, are usually biologically inactive. Even when the alterations are specifically directed and well controlled as in the formation of N-acyl or perhydro derivatives of AB, the products may be poorly active[2,9]. These findings serve to underline the importance of stereospecificity in the reaction of polyene antibiotics with membrane sterols which forms the basis of mode of action of the antibiotics. It is interesting that despite repeated attempts, the only derivatives of polyene antibiotics yet to be synthesized that exhibit a full retention of antifungal activity have been the ester derivatives formed at the free carboxyl group.

When we consider the non-specific reaction possibilities able to be induced by heat or acid on such a complex structure as AB, it is easy to see why storage in such an environment can lead to a rapid loss of biological activity. In the case of AME, the problems concerning stability have only been compounded by the altered physical properties of this derivative. In most systems, AB is either poorly soluble or exists as a colloidal dispersion. Under aqueous conditions, with which we have concerned ourselves in these studies AB exists in the form of large micelles consisting of many molecules. We feel that this micellar formation, due to hydrophobic and hydrophilic forces, may confer a degree of protection on AB in that the sensitive regions of the molecule may be partially shielded from adverse elements within the environment. On the other hand it has been shown in spectrophotometric studies[3] that AME is dispersed to a far greater degree than AB when in an aqueous system. With its increased solubility and increased accessibility to chemical reaction, the effect of heat and acid on the stability of AME is more pronounced than with AB.

The consideration of aerobic degradation of AME prompted us to compare the relative stabilities of the hydrochloride and ascorbate salts of this derivative. It had been observed for the pentaene macrolide antibiotic, filipin, that storage in the absence of air greatly improved the stability of this compound[10]. The anti-oxidant properties of ascorbic acid are recognized and it was felt that the use of this acid in salt formation may also limit the aerobic breakdown of AME. In our short term studies we observed no significant differences in the stabilities of the hydrochloride and ascorbate salts of AME. We feel, however, that in long term situations, where air is not excluded, storage of AME as the ascorbate salt may be advantageous.

The purpose of our investigation has not only been to investigate but to define storage conditions that insure an adequate stability for amphotericin B methyl ester. We have observed that the presence of acid and elevated temperature enhance the degradation of AME.

However, when the pH of the AME salt is brought near neutrality and storage temperature is held at normal refrigeration conditions (+5°C), AME is similarily stable to its parent compound AB.

Acknowledgments

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