The polyene macrolide antibiotics

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Summary

The mode of action of the polyene antibiotics is reviewed together with the effect of genetic and environmental factors on sensitive organisms. The future prospects of polyenes in the treatment of systemic mycoses are considered.

THE effective treatment of any infection requires at least one significant difference between host and parasite to provide a target for the chemotherapeutic agent. Fungi, like their human hosts, are eukaryotes and the differences which could provide a target are limited and occur largely in the cell envelope. Numerous antifungal antibiotics have been isolated but most are toxic and few are used clinically. The polyene macrolide antibiotics comprise a complex group of some 200 compounds produced by various *Streptomyces* spp. of which only amphotericin B is sufficiently non-toxic to be used to treat systemic mycoses. Chemically these compounds are characterized by a carbon ring possessing both a system of conjugated double bonds and a hydrophilic region and closed by a lactone bond. Polyenes differ in the number of carbon atoms in the ring, the number of conjugated double bonds and the number of hydroxyl residues (Hamilton-Miller, 1973; Cartwright, 1975; Thomas, 1976; Norman, Spielvogel and Wong, 1976; Kobayashi and Medoff, 1977). The complete structure of amphotericin B has been elucidated by Mechlini *et al.* (1970) and this antibiotic is an elongated rigid molecule with opposing hydrophobic and hydrophilic faces, features of undoubted importance in its biological activity.

The polyenes interact with sterol-containing membranes of eukaryotic organisms causing impairment of function and ultimately cell death; prokaryotic organisms, other than sterol-containing *Acholeplasma* spp. and *Mycoplasma* spp., are unaffected (Lampen, 1966). The extent of membrane damage depends both on the lipid composition (in particular the sterols) of the membrane and the antibiotic (Norman *et al.*, 1976). In general, polyenes with a lower molecular weight cause more damage

than those with a higher molecular weight. The differences in the affinities of polyenes for cholesterol-containing mammalian membranes and ergosterol-containing fungal membranes make it possible to use certain of these antibiotics in the chemotherapy of mycotic infections (Gale, 1973; Archer and Gale, 1975).

The mechanisms by which individual polyenes interact with the membranes of sensitive cells may differ from one polyene to another. In the case of amphotericin B (a heptaene) and nystatin (a tetraene) data, derived largely from *in vitro* experiments with artificial membranes, support the hypothesis that incorporation of the polyene into the membrane results in the production of aqueous pores consisting of an annulus of polyene and sterol in which the hydrophilic region of the polyene faces the interior of the pore. The length of the annulus is such that 2 half pores would appear necessary to span the membrane (De Kruijff and Demel, 1974; Andreoli, 1973; Holz, 1974). Marty and Finkelstein (1975) have proposed a modification where either a single or double annulus can span the bilayer in each case with distortion in the vicinity of the polyene. This model may help to explain the production of pores in the membranes where the antibiotic is added to the outside of sensitive organisms. The polyene-membrane interaction is reversible both *in vitro* (Holz, 1974) and *in vivo* (Cass and Dalmark, 1973; Kumar *et al.*, 1974; Liras and Lampen, 1974; Kerridge, Koh and Johnson, 1976 a) and the inhibitory effects would appear to result from the loss of K⁺ ions and other low molecular weight constituents (Lampen, 1966). This hypothesis is supported by the findings that for *Saccharomyces cerevisiae* (Liras and Lampen, 1974) and *Candida albicans* (Kerridge *et al.*, 1976 b) the inhibitory effects of candidin and amphotericin B respectively are prevented by the concomitant addition of K⁺ and Mg⁺⁺.

However, this hypothesis requires revision in the light of recent experimental findings. *In vitro* studies by Hsu-Chen and Feingold (1973) and *in vivo* studies by Archer (1976) have provided evidence

that the membrane fluidity is of primary importance and the presence of the sterol modulates this fluidity. Polyene-induced K^+ leakage has been widely used as a measure of the interaction of the antibiotic with sensitive organisms (Hammond and Kliger, 1974; Gale, 1974; Johnson, White and Williamson, 1978) but there is evidence that the loss of intracellular K^+ may not be responsible for cell death. Polyene-resistant mutants of *C. albicans* have been isolated in which polyene-induced leakage of low molecular weight cellular constituents occurs under conditions where there is no loss of viability (Hsu-Chen and Feingold, 1974). Hammond, Lambert and Kliger (1974) and more recently Chen, Chou and Feingold (1978) have demonstrated a dissociation of K^+ ion permeability and the lethal action of the polyene antibiotics and the latter authors suggest that the aqueous pores formed by these antibiotics are not central to their lethal action. Further evidence that K^+ leakage may not be directly responsible for the loss of viability comes from studies by Palacios and Serrano (1978) on the amphotericin B inhibition of maltose fermentation in *S. cerevisiae* where they demonstrated a polyene-induced increase in proton permeability. Since K^+ accumulation in yeast is linked to the proton gradient (Pena, 1975) this could account for the polyene induced K^+ leakage (see also Hugo, 1978). Finally Solov'eva, Belousova and Tereshin (1976) have shown an inhibitory effect of polyenes on the activity of certain membrane-bound enzymes of *C. albicans*, but the relationship of this to the loss of viability is not clear.

The interaction of polyenes with sensitive organisms is affected by genetic and environmental factors. Amphotericin B may have severe side effects when administered and the maximum concentration attainable in the serum is of the order of 1–3 $\mu\text{g/ml}$. In a study of clinical isolates of *C. albicans*, Hamilton-Miller (1972) found that 17% of them were not inhibited by amphotericin B at concentrations normally found in serum and it is clear from these data that even small variations in the sensitivity of these organisms could be clinically important. The occurrence of amphotericin-resistant mutants of *C. albicans* is not a serious clinical problem and most studies of genetically mediated resistance have been undertaken with mutagen-treated cultures. In these strains, resistance appears to be associated with an alteration in the membrane sterols resulting from a block in ergosterol biosynthesis and an increase in the total sterol content (Pierce *et al.*, 1978). It is interesting to note that in this context Virina *et al.* (1976) found no differences in the binding of amphotericin B to polyene-sensitive and to resistant strains of *C. albicans*.

The interaction of amphotericin B with *C. albicans* is influenced by a variety of environmental factors.

The addition of sterols together with the polyene can reduce its effect on the growth of *C. albicans* (Archer and Gale, 1975) and this apparent increase in the minimum growth inhibitory concentration results from the lowering of the effective concentration due to an *in vitro* interaction between the polyene and lipid (Bittman and Fishkoff, 1972; Norman *et al.* 1972). The presence of lipids in the growth medium also affects the composition of the cellular membranes of an oleic acid-requiring strain of *C. albicans* and, as a result, the sensitivity of this organism to inhibition by amphotericin B (Koh *et al.*, 1977). The inhibitory effects of certain polyenes can also be prevented by the concomitant addition of K^+ and Mg^{++} (Liras and Lampen, 1974; Kerridge *et al.*, 1976 a). In this case the antibiotic interacts with the plasma membrane but the internal concentration of ions is maintained and growth continues.

Phenotype variation in *C. albicans* also affects the antibiotic sensitivity of this organism; not only are there changes in the sensitivity to amphotericin B after the cessation of growth (Hammond and Kliger, 1974; Gale, 1974) but also in cultures grown in continuous culture under different environmental conditions (Johnson *et al.*, 1978). In these studies polyene-mediated K^+ release was used to monitor sensitivity to polyenes but since the authors used different techniques the results are not directly comparable. After the cessation of growth of *C. albicans* the organism becomes increasingly resistant to amphotericin B methyl ester. This increased resistance results from changes in the cell wall since sphaeroplasts derived from resistant organisms are sensitive (Gale, 1974). The development of resistance is affected by a variety of factors including complexity and pH value of the growth medium, degree of aeration and presence of metabolic inhibitors (Gale, Johnson and Kerridge, 1977; Gale *et al.*, 1975, 1978). The sensitivity of the organism can also be altered by chemical or enzymatic treatment of the cell wall. Ultrastructural studies of the cell wall of *C. albicans* have shown that after 72 hr's starvation, when resistance to amphotericin B methyl ester had increased some 60-fold, both the characteristic layering and the periplasmic material were lost. There was however no change in thickness of the wall at this stage although after 144 hr the wall had increased in thickness to 211 ± 58 nm from the initial value of 143 ± 22 nm (Cassone, Kerridge and Gale, 1978). Although there are considerable changes in the ultrastructure of the cell wall on starvation it has not been possible to detect any significant differences in composition of the cell walls isolated from resistant and sensitive cultures (Kerridge *et al.*, 1976 b), but this might be due to loss of material during preparation (Cassone *et al.*,

1978). However, given the complexity of the cell wall of *C. albicans* and the size and shape of the polyene molecule it is perhaps not surprising that even small variations in structure and organization of the cell wall can profoundly affect the interaction of the antibiotic with the underlying plasma membrane.

What are the future prospects for polyenes in the treatment of systemic mycoses? the major problem is that we are dealing with a eukaryotic pathogen in a eukaryotic host and differences between them reside largely in the cell envelope, with the pathogen having a complex cell wall surrounding an ergosterol-containing plasma membrane and the host having a cholesterol-containing membrane with no cell wall. These features are important in both the selectivity and the development of antibiotic resistance in the pathogen; and clearly one possibility for the future would be to search for new polyenes or derivatives of existing ones with a greater affinity for ergosterol-containing membranes (and preferably fewer side reactions) and whose activity is less affected by the phenotypic changes which can occur in the cell wall. Polyenes are large, complex and unstable molecules and the probability of a successful chemical or bio-synthetic modification low. However, there has been one notable success in the synthesis of the methyl ester of amphotericin B (Mechlinski and Schaffner, 1972; Chen *et al.*, 1977) where methylation has increased the apparent solubility and reduced toxicity without reducing the antifungal activity, and clearly this approach must be pursued.

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