COMPARATIVE CHEMOTHERAPEUTIC ACTIVITIES OF HEPTAENE MACROLIDE ANTIFUNGAL ANTIBIOTICS IN EXPERIMENTAL CANDIDIASIS

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Nine heptaene macrolide antifungal antibiotics, amphotericin B, azacolutin, candicidin, hamycin, heptamycin, levorin, mycoheptin, perimycin, and trichomycin were active against a systemic *Candida albicans* infection of mice. While all compounds exhibited similar activity when administered subcutaneously, only amphotericin B and mycoheptin showed significant activity when given orally. This high level of oral activity in mice is probably a characteristic of the group of heptaenes that contain only mycosamine as a nitrogenous moiety and thus should extend also to the candidin complex. Although amphotericin B is inactive when given orally to man, mycoheptin, candidin, or a chemically-modified heptaene might show useful therapeutic efficacy when given to man by the oral route.

In an earlier report[1] we compared the therapeutic activities of two heptaene macrolide antifungal antibiotics, amphotericin B and hamycin, when these were administered by the oral route to mice infected with *Candida albicans*. Amphotericin B proved to be far more effective than hamycin. We had long known that amphotericin B, in this model infection, was approximately three times more effective when administered subcutaneously than when given orally. In further studies with hamycin, we were surprised to find that this same relationship did not hold with this second antibiotic. We, therefore, decided to study as many members of the heptaene antifungal group as were available to us, in an effort to account for this difference.

Materials and Methods

Candida albicans, Squibb Culture Collection, Strain #5314, was grown on the surface of SABOURAUD'S dextrose agar slants for 48 hours at 37°C. Growth was washed off with 0.85% NaCl solution and diluted to give 10^6 cells per ml by the use of a Bauch & Lomb photoelectric colorimeter at 550 mμ and a curve derived from plate counts. CF1 male mice, 18~20 g, were infected by injection of 0.5 ml (5×10^5 viable units) of the culture suspension into a lateral tail vein. The mice were pooled in a community cage and then assigned randomly to pans in groups of ten. The mice were treated with single doses of antibiotic, either by stomach tube or subcutaneously, 1 hour after infection.

The death times for all mice were recorded until the death of the last untreated control animal, at which time the experiment was terminated. The median protective doses (PD_{50}) were calculated by the method of REED and MUENCH[2]. Control mice usually died between the second and seventh day.

All antibiotics were dissolved in pharmaceutical grade dimethylsulfoxide and then diluted in distilled water to concentrations well below the toxic level of the solvent. Amphotericin B, suspended in this manner, and amphotericin B-sodium desoxycholate mixture[3] which is readily dispersed colloidally in water, showed identical activities. The antibiotics employed were:

Amphotericin B, (Batch #38675-001, assay 991 μg/mg) E. R. Squibb & Sons.
Azacolutin, (Batch PL 145-1), Dr. A. Aszalos, E. R. Squibb & Sons.
Candicidin, (Lot 8482-NHF-1, assay 2,090 μg/mg) Julius Schmid, Incorporated.
Hamycin-Sample 1, (Lot 7213) Sherman Laboratories.
Hamycin-Sample 2, Dr. C. W. Emmons, National Institutes of Health.
Heptamycin, (40,000 units/mg), Dr. N. Grossowicz, Hebrew University-Hadassah Medical School, Jerusalem, Israel.
Levorin, Institute of Antibiotics, Leningrad, U.S.S.R.
Mycoheptin, (650 μg/mg), Institute of Antibiotics, Leningrad, U.S.S.R.
Perimycin, (90 % pure), Dr. C. Schaffner, Institute of Microbiology, Rutgers University, New Brunswick, NJ.
Trichomycin, (Lot #1607-2, 7,620 units/mg), Fujisawa Pharmaceutical Co.

Results and Discussion

The results show that all nine heptaenes were therapeutically effective by both the subcutaneous and oral routes (Table 1). In the case of levorin, the dose of compound required for oral activity was very close to the toxic level. Until now, amphotericin B[4] and hamycin[1] are the only heptaenes that have been reported as effective against a *C. albicans* infection in mice when given orally. Candicidin has been reported as inactive against this infection in mice when given by the oral route[5,6] However, examination of the data presented in the latter paper suggests that the criterion for activity may have been set too high, since high oral doses of candicidin increased the mean survival time of infected mice from 1.9 days to 5.4 days.

The ratio of subcutaneous PD_{50} to oral PD_{50} varied widely from one antibiotic to another. It is very probable that there were considerable differences in the purities of some of the antibiotics. If we acknowledge such differences then all heptaenes showed approximately the same order of activity when administered subcutanously. That the variation in purity was considerable is obvious from the differences in activities that were found for the two samples of hamycin. Amphotericin B and mycoheptin differed from the other seven heptaenes in having closely related oral and subcutaneous PD_{50}'s.

Table 1. The activities of several heptaene macrolide antifungal antibiotics when administered orally and subcutaneously to mice infected systemically with *Candida albicans*

Antibiotic	PD_{50} (mg/kg) when administered		Ratio of PD_{50}'s Oral/Subcutaneous
	Orally	Subcutaneously	
Amphotericin B	0.43	0.11	3.9
Azacolutin	59.0	0.11	537
Candicidin	85.0	0.46	185
Hamycin-Sample #1	14.3	0.24	60
Hamycin-Sample #2	53.6	0.62	86
Heptamycin	442	3.6	123
Levorin	234	1.0	234
Mycoheptin	0.83	0.27	3.4
Perimycin	220	0.57	387
Trichomycin	290	1.67	173

The relationship found between the oral and subcutaneous activities of amphotericin B and mycoheptin agrees with their grouping by Borowski and Schaffner[7] and Lee and

SCHAFFNER[8] in the chemical classification of the heptaene antifungals. These workers classified the heptaene antifungals on the basis of the nitrogenous moieties found after acid and alkali hydrolyses. In the beginning, their group of compounds containing mycosamine, consisted only of amphotericin B and candidin. Later mycoheptin, candidinin, and candidoin were added to this group[9]. To their aromatic group 2a, which contained compounds possessing mycosamine and *p*-aminoacetophenone, belonged candicidin and trichomycin. Later ascosin, trichomycins A and B[9], levorin[10], and hamycin[11] were added to this group. A third aromatic group, 2b, limited to compounds possessing perosamine and N-methyl-*p*-aminoacetophenone, contained only perimycin. A fourth aromatic group has been proposed for the heptaene aureofungin[12], this group comprising compounds that possessed mycosamine and N-methyl-*p*-aminoacetophenone.

All of the heptanes we have studied are included in the above groupings except for azacolutin and heptamycin. As far as we know, these two heptaenes have not been studied for their nitrogenous compostion.

Opinion varies as to whether candicidin, trichomycin, levorin, and hamycin are actually different substances. It has been suggested that hamycin and trichomycin are identical[13], and that candicidin is identical with trichomycin[14]. Recently it was suggested[15] that candicidin, levorin, and trichomycin are mixtures with a single identical main component and varying proportion of similar minor components. Further biological studies may reveal some differences among several members of this group.

It is apparent that the chemical grouping of amphotericin B and mycoheptin is mirrored by the high therapeutic activities they show when administered orally to mice. It would be interesting to study the other members of this same classification group, candidin, candidinin, and candidoin to see if the high oral activity displayed by amphotericin B and mycoheptin is truly a group characteristic. We would predict that candidin, at least, would show this characteristic, for in addition to their chemical relationship, it has been shown that candidin and amphotericin B have almost identical antifungal spectra *in vitro*, differing considerably from the spectrum of candicidin[16].

The excellent therapeutic activity of amphotericin B seen after oral administration in mice has not been translated to man[17]. It would be interesting to determine if mycoheptin (and also candidin) are active orally in man, or to attempt chemical modification of some heptaenes in the hope of producing potent, useful orally active antifungal agents.

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