POLYENE MACROLIDE DERIVATIVES. III

BIOLOGICAL PROPERTIES OF POLYENE MACROLIDE ESTER SALTS

Sir:

The polyene antibiotics have been characterized as potent antifungal agents with limited clinical applicability due to their great toxicity[1,2]. With the development of a new derivative of the polyene macrolides, namely, the soluble methyl ester salt[3,4], a program of chemotherapeutic evaluation was initiated.

In vitro activity of several different polyene macrolides and their methyl ester hydrochloride salts against a *Saccharomyces cerevisiae* culture was determined using the agar dilution method. The results shown in Table 1 indicate almost complete retention of antifungal activity by the soluble derivative as compared to the parent compound.

Acute intraperitoneal toxicities of four of the polyene macrolides and their derivatives are shown in Table 2. The animals used were HA-ICR female mice, 20~22 g. The antibiotics were suspended in sterile 5% dextrose solution. Each mouse received 0.25 ml of the preparation. Deaths were recorded daily and tabulated after 10 days.

Table 1. *In vitro* activity of polyene macrolides and their methyl ester hydrochlorides against *Saccharomyces cerevisiae* # 216

Compound	M.I.C. (μg/ml)
Nystatin (tetraene)	3.00
Nystatin methyl ester hydrochloride	4.00
Pimaricin (tetraene)	3.50
Pimaricin methyl ester hydrochloride	4.00
Mediocidin (hexaene)	0.035
Mediocidin methyl ester hydrochloride	0.030
Amphotericin B (heptaene)	0.25
Amphotericin B methyl ester hydrochloride	0.25
Candicidin (heptaene)	0.020
Candicidin methyl esther hydrochloride	0.025
Trichomycin (heptaene)	0.030
Trichomycin methyl ester hydrochloride	0.030
Candimycin (heptaene)	0.025
Candimycin methyl ester hydrochloride	0.025

The polyene macrolide methyl ester derivatives show a uniform decrease in toxicity when compared with their parent compounds.

Acute intravenous toxicity in mice was evaluated for one of these antibiotics. Amphotericin B methyl ester hydrochloride (AME) demonstrates an intravenous LD_{50} in excess of 75 mg/kg, while Fungizone(R), a clinical formulation of amphotericin B utilizing sodium desoxycholate as a solubilizing agent, demonstrates an intravenous LD_{50} of 4.5 mg/kg (based on amphotericin B content)[5].

To evaluate the *in vivo* antifungal activity of AME, mice infected with *Candida albicans* were used. Fungizone was used as a control drug. Groups of mice were infected intravenously with one million cells of *Candida albicans* on day 0. This inoculum was sufficient to yield a median survival time of 9 days in the control groups. Other groups of infected mice were treated with either Fungizone or AME in various concentrations. Drugs were administered intraperitoneally on days 1, 3, 5, 7, and 9. The 21-day results are shown in Table 3.

Both drugs seem to be quite effective at the levels of 10 mg/kg and 1 mg/kg. At the level of 0.1 mg/kg both drugs demonstrate marginal efficacy. From the limited

Table 2. Acute intraperitoneal toxicities of polyene macrolides and their methyl ester hydrochlorides in mice

Compound	Dose mg/kg	No. of deaths
Amphotericin B*	99	4/4
Amphotericin B methyl ester hydrochloride	300	0/4
	400	2/4
	600	4/4
Candicidin	15	3/4
	20	3/4
Candicidin methyl ester**	15	0/4
	20	0/4
Candidin	10	3/4
	15	4/4
Candidin methyl ester hydrochloride	10	0/4
	20	0/4
	40	2/4
Mediocidin	10	2/4
Mediocidin methyl ester hydrochloride	10	0/4

* Administered as Fungizone.
** Administered as free base.

Table 3. Comparison of *in vivo* activity of amphotericin B methyl ester hydrochloride and Fungizone against *Candida albicans* #204 in mice.

Antibiotic	Dose mg/kg/day	No. of deaths at 21 days
Amphotericin B methyl ester hydrochloride	10	0/6
	1	1/6
	0.1	4/6
	0.01	5/6
Fungizone	10	0/5
	1	0/6
	0.1	2/6
	0.01	4/6
Control	—	10/12

data, it appears that AME may be slightly less effective than Fungizone in controlling the *Candida albicans* infection in mice.

Since the polyene macrolides have been shown to possess antiprotozoan activity[6,7], AME was evaluated against a *Plasmodium berghei* infection in mice. Preliminary indications are that the methyl ester hydrochloride of amphotericin B is successful in significantly prolonging the life of mice infected with *Plasmodium berghei* as compared to control groups. While infected control groups demonstrated 100 % mortality within 8 days, 60 % of the group treated intraperitoneally with AME (75 mg/kg×5 daily doses) survived until day 12. The administration of similar high concentrations of amphotericin B necessary to achieve this effect, would have been prohibitive due to great inherent toxicity of the parent compound.

Acknowledgements

We acknowledge the support of the National Institute of Allergy and Infectious Diseases under Public Health Service Grant No. AI-02095 and of the National Institute of General Medical Sciences under Public Health Service Subcontract No. 69-2161. D. P. BONNER was supported by the National Institute of General Medical Sciences Training Grant 5T01 GM 507.

The authors thank Dr. M. SOLOTOROVSKY and Dr. L. STAUBER of Rutgers University for providing the cultures of *Candida albicans* and *Plasmodium berghei*, respectively, and for their technical advice and also Dr. WILLIAM BROWN of Squibb Institute for Medical Research for a gift of the Fungizone used in these studies.

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(Received February 1, 1972)

References

1) WAKSMAN, S. A.; H. A. LECHEVALIER & C. P. SCHAFFNER: Candicidin and other polyenic antifungal antibiotics. Bull. World Health Org. 33: 219~226, 1965
2) LAMPEN, J. O.: Amphotericin B and other polyenic antifungal antibiotics. Amer. J. Clin. Path. 52: 138~146, 1969
3) MECHLINSKI, W. & C. P. SCHAFFNER: Polyene macrolide derivatives. I. N-Acylation and esterification reactions with amphotericin B. J. Antibiotics 25: 256~258, 1972
4) SCHAFFNER, C. P. & W. MECHLINSKI: Polyene macrolide delivatives. II. Physical-chemical properties of polyene macrolide esters and their water soluble salts. J. Antibiotics 25: 259~260, 1972
5) BARTNER, E.; H. ZINNES, R. A. MOE & J. S. KULESZA: Studies on a new solubilized preparation of amphotericin B. Antibiotics Ann. 1957/1958: 53~58, 1958
6) SENECA, A.: *In vivo* and *in vitro* effect of nystatin on *Entamoeba histolytica*. Antibiotics Ann. 1955/1956: 697~703, 1956
7) STAUBER, L. A.: Some recent studies in experimental leishmaniasis. Sci. Repts. Ist. Super·Sanita 2: 68~75, 1962