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Potentiation of Amphotericin B Activity Against *Trypanosoma congolense* in Mice (27532)

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The antifungal activity of the polyenic antibiotics produced by streptomycetes is well documented. Amphotericin B is of significant value in treatment of a wide variety of systemic and topical mycotic infections of man and animal. In addition to its antifungal activity this polyene has been shown to be effective against experimental infections with several parasitic protozoa(1-6). Stauber and his co-workers(2-3) demonstrated that amphotericin B possessed marked therapeutic activity against *Leishmania donovani* infections of hamsters and mice. Recent clinical studies have confirmed this laboratory evidence(7-9). This report is concerned with the effect of amphotericin B on another protozoan parasite, *Trypanosoma congolense,* and with the possible mode of action of this antibiotic.

Materials and methods. Male CF_1 mice weighing approximately 20 g were employed in all *in vivo* experiments. The mice were infected either subcutaneously or intraperitoneally with 0.5 to 2.5 $\times$ 10^6 trypanosomes contained in blood obtained from donor mice.

Amphotericin B as Fungizone for Infusion® is complexed with sodium desoxycho-

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TABLE I. *In vitro-in vivo* Effect of Amphotericin B against *T. congolense*.

Drug	$\mu g/5 \times 10^5$ organisms	Survivors/Total
None	—	0/30
Amphotericin B	.5	0/10
"	2.5	1/10
"	5.0	8/10
"	25.0	10/10
"	50.0	10/10
Na desoxycholate	.5	0/10
"	2.5	0/10
"	25.0	0/10
"	50.0	0/10

late resulting in a colloidal dispersion which is suitable for intravenous administration. Thorium dioxide was administered in a 20% suspension plus 1.6% dextrin. Triamcinolone acetonide (Kenalog®) was used as an aqueous suspension. India ink (Pelikan, Gunther Wagner, Germany) was injected as an autoclaved 10% suspension containing 1% Difco gelatin.

Since *T. congolense* cannot be grown in culture, a simple test was employed to determine *in vitro* activity. Mixtures of drug and parasites obtained from the blood of donor mice were held at room temperature for 5 minutes prior to intraperitoneal inoculation into mice. The survival pattern of the infected mice was then used as the criterion for evaluation of the drug.

Results. Exposure of blood suspensions of *Trypanosoma congolense* to graded concentrations of amphotericin B and subsequent intraperitoneal injection of the drug-parasite mixture into mice affected the course of infection in these mice (Table I). Levels as low as 2.5 $\mu g/5 \times 10^5$ organisms altered the survival pattern of the infected mice. A level of 0.5 μg extended the survival time, but did not allow survival, whereas 100 times this level of sodium desoxycholate had no effect on the parasite.

Intraperitoneal administration of amphotericin B (4 doses over a 4-day period, starting 24 hours prior to infection) revealed only limited activity as evidenced by a significant extension of survival time of the treated mice (Table II). When administered intravenously, amphotericin B failed to demonstrate significant activity.

Tables III and IV summarize the results obtained when the reticuloendothelial system (RES) was blocked with a number of different agents prior to intravenous amphotericin B treatment. Table III represents 2 experiments, the data of which were pooled because of their similarity. It can be seen from these tables that all of the blocking agents, in combination with amphotericin B, extended survival time of the infected mice. The combination of thorium dioxide and triamcinolone acetonide offered the most potent enhancement of amphotericin B activity.

TABLE II. Effect of Intraperitoneal Administration of Amphotericin B against *T. congolense*.

	Change in median survival time (hr)	
mg/kg/day	Infected IP*	Infected subcut.*
.05	− 4	NT
1.00	+ 22	NT
5.00	+ 80	NT
12.50	NT	+ 5
25.00	+118	+21

* Median survival time of intraperitoneal (IP) infected controls was 72 hr; subcutaneous infection (subcut.) 96 hr.

NT = Not tested.

Discussion. Amphotericin B was shown to possess a high order of activity against *Trypanosoma congolense in vitro* (Table II). Despite this potent trypanocidal effect, this antibiotic gave only limited activity when administered parenterally (Table I). The best *in vivo* activity was observed when drug and parasite were introduced by the same route, allowing for greater access of drug to organism.

TABLE III. Potentiation of Activity of Amphotericin B *via* Blockade of the RES with India Ink.

		% survival		
		Hr post infection		
Treatment	No. mice	117 hr	141 hr	173 hr
None	24	17	0	0
India ink*	26	19	0	0
Amphotericin B†	16	19	0	0
India ink plus amphotericin B	17	53	12	0

* .5 ml of a 10% suspension administered intrav. 24 and 2 hr prior to infection.

† 4 mg/kg administered intrav. 2, 24, 48 and 72 hr post infection.

TABLE IV. Potentiation of Activity of Amphotericin B *via* Blockade of the RES with Thorium Dioxide and Triamcinolone Acetonide.

Drug	Blocking agent	No. mice	% survival, Hr post infection 117 hr	141 hr	173 hr
None	None	20	0	0	0
Amphotericin B*	"	20	25	5	0
None	Thorium dioxide†	15	0	0	0
"	Triamcinolone acetonide‡	14	0	0	0
Amphotericin B	Thorium dioxide	17	59	24	6
"	Triamcinolone acetonide	14	93	50	29
"	Thorium dioxide + triamcinolone acetonide	12	100	92	67

* 4 mg/kg intrav., 2, 24, 48, and 72 hr post infection.
† 0.2 ml of a 10% suspension intrav., 2 hr prior to infection.
‡ 20 mg/kg subcut., 72 and 48 hr prior to infection.

Approximately 90% of amphotericin B is removed from the blood within 15 minutes after intravenous administration(10). It is quite possible that the phagocytic cells of the reticuloendothelial system (RES) are partly responsible for this rapid removal of amphotericin B. This would in part explain the remarkable activity of this antibiotic in the animal against several intracellular organisms such as *Leishmania donovani* and *Histoplasma capsulatum,* in that the same cells responsible for removing amphotericin B harbor the parasite. The effectiveness of amphotericin B therefore might be due to the proximity of drug and parasite within the cells of the RES.

T. congolense multiplies only in the blood and does not reside in the cells of the RES. It was reasoned that rapid phagocytosis or pinocytosis of amphotericin B, and the resulting low blood levels, might prevent it from acting on the parasite. If this were true, it follows that an effective blockade of the RES would prevent the uptake of amphotericin B and result in higher levels of the drug remaining free in the blood.

Although none of the blocking agents *per se* altered the survival pattern of the infected mice, they all potentiated the activity of amphotericin B (Tables III and IV). High levels of steroid have been reported to inhibit RES function(11-12). The functional blockade induced by triamcinolone acetonide apparently was superior to that of the physical agents employed.

The experiments described suggest several interesting implications. Phagocytosis and/or pinocytosis of amphotericin B by the RES may partly explain the poor recovery of this antibiotic from the blood following intravenous administration, and may also explain its occasional failure in disseminated mycotic infections. On the other hand, the activity of this polyene against intracellular parasites such as *H. capsulatum, L. donovani* and *T. cruzi* may be enhanced by its insoluble nature. Temporary blockade of the RES with a suitable blocking agent may be a means to increasing the levels of amphotericin B free in the blood.

Summary. Amphotericin B was shown to have a high order of *in vitro* activity against *T. congolense,* whereas only limited activity was observed *in vivo.* The limited activity was attributed to the rapid phagocytosis of amphotericin B by cells of the reticuloendothelial system (RES) with resulting lowered concentration free in the blood stream. Marked potentiation of activity was observed when intravenous treatment with amphotericin B was preceded by the blockade of the cells of the reticuloendothelial system (RES) with suitable agents such as thorium dioxide or triamcinolone acetonide.

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Effects of Two Erythrophleum Alkaloids on Potassium Transfer in Human Erythrocytes.* (27533)

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Alkaloids isolated from the bark of *Erythrophleum guineense* have been known since 1875(1). Maling and Krayer(2) found that the erythrophleum alkaloids cassaine, norcassaidine, erythrophleine, and coumingine produced effects in the dog heart-lung preparation which were similar in some respects (increased contractile force and production of irregularities) to those produced by the cardiac glycosides. Further investigations (for a summary, see 3) have supported these conclusions. The chemical relationships of cassaine and coumingine to cassaic acid have been known for some time (cf. 2); recently the structure of cassaic acid has been demonstrated by Turner *et al.*(4). These structures are shown in Fig. 1. In contrast to the cardiac glycosides, the erythrophleum alkaloids have no cyclopentane ring fused to the phenanthrene nucleus, nor do they have a lactone ring in the molecule. Two other features thought necessary for glycoside-like effects (5), a C-14 –OH group and a cis-fusion of the A and B rings, are also lacking in the erythrophleum alkaloids. Since there is evidence indicating that the latter have cardiac effects like those of the glycosides, the present experiments were done to determine whether the 2 kinds of compounds also share a non-cardiac effect previously unique to glycosides and their lactones, *i.e.,* inhibition of active K accumulation by human erythrocytes (RBC).

Methods. Blood was obtained and treated as previously described(6). Cassaine sulfate and coumingine hydrochloride were dissolved in K-free 0.9% NaCl, and added to 4.2-4.9 ml of blood in volumes up to 0.1 ml. Control flasks received an equal amount of 0.9% NaCl. $K^{42}Cl$ was added in concentrations less than 0.05 meq/l blood; radioactivity was such as to produce counting rates of 1-3 $\times 10^4$ cpm per ml of plasma. Incubation was for 3 hours at 37°. Before and after incubation, measurements were made of whole blood and plasma K^{42} in the well of a scintillation counter, and total K in a Baird KY-1 flame photometer. Micro-hematocrits were done in duplicate. Changes of K from plasma to cell over a 3-hour period were estimated from calculations of specific activities and measurements of K^{42}.

Results. In 9 experiments, the initial concentration of plasma K was 5.24 ± 0.28 (S. E.M.) μeq/ml plasma. The mean control rate of K transfer into the RBC during 3 hours of incubation was 4.98 ± 0.22 μeq K/ml RBC/3 hours; this K exchange rate is similar to those reported previously (cf. 6).

The data obtained with the alkaloids are given in Fig. 2. Both cassaine and coumingine could inhibit active K accumulation by

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