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## **Treatment of experimental cutaneous leishmaniasis by liposome-entrapped Pentostam**

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### **Introduction**

The ability of liposomes (small phospholipid vesicles) to be taken up by cells of the reticulo-endothelial system makes them ideal vehicles for the selective transport of drugs to target tissues in diseases where the phagocytic cells are a centre of infection. This has been demonstrated in the case of experimental infections of visceral leishmaniasis where antileishmanial compounds inside liposomes such as potassium antimony tartrate and sodium antimony gluconate have been brought into close contact with amastigotes in liver Kupffer cells resulting in complete clearance of parasites from the liver at much lower doses than is possible with the free drug alone (Alving et al., 1978; Black et al., 1977; New et al., 1978).

### **Materials and methods**

Egg yolk lecithin, cholesterol and dicetyl phosphate (DCP) were obtained from BDH Chemicals Ltd. The lecithin (PC) was purified by passing through a neutral alumina column to give a single spot on T.L.C. No further purification of the other lipids was required. Sodium antimony gluconate (Pentostam) was purchased from Burroughs Wellcome as a concentrated solution with preservatives, or received as a pure powder as a gift from the Wellcome Foundation.

#### *Preparation of liposomes*

Liposomes were prepared after the method of Bangham and Horne (1964) as previously described (New et al., 1978). Lipids were dried in a rotary evaporator in the following ratio: 200 mg PC : 25 mg cholesterol : 25 mg DCP and dispersed in 10 ml distilled water containing 2 g Pentostam by means of an MSE 150 watt Ultrasonic Disintegrator for a total of 5 min, with cooling. Pentostam not entrapped inside the liposome vesicles during their formation was removed by dialysis for four days against daily changes of 2 litres distilled water at 4° C. The concentration of entrapped pento-

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stam remaining in the liposome suspension was measured by an EEL 240 atomic absorption spectrophotometer. The liposomes were concentrated if necessary by centrifugation at 200,000 g and the pellet resuspended in distilled water to give a final concentration of Pentostam of 5 mg/ml.

#### *Model for cutaneous leishmaniasis*

10<sup>6</sup> promastigotes of *Leishmania major* (strain P, LV 39) obtained as a primary isolate from infected tissue were inoculated subcutaneously at a shaven area at the base of the tail into adult TFW outbred mice. The severity of the lesions developing at the site of inoculation was assessed by macroscopic observation on a scale of 1 to 4 as follows:

- 1) thickening of skin
- 2) nodule <5 mm
- 3) nodule >5 <7 mm
- 4) nodule or ulcer >7 mm.

The scores within each group of five mice were added together and tabulated as the variation of the score with type of treatment.

Treatment was carried out over a five-day period with a dose of 50 mg/kg Pentostam being administered per day by various routes.

## Results

In our initial experiments animals were treated immediately after inoculation of the parasites, a gap of three hours elapsing between the promastigote inoculation and the first dose in the five-day course of drug treatment. Table 1 shows the effect of treatment with Pentostam, both as free drug and encapsulated within liposomes, by different routes on the development of cutaneous lesions nine weeks after the start of the experiment. Liposome-entrapped Pentostam is compared with the same dose of Pentostam administered in the free state, and it is clear in the intravenous administration that liposomes enhanced

Table 1. Severity of cutaneous lesions in mice nine weeks after inoculation of promastigotes of species *L. major*. Effect of treatment with free and liposome-entrapped Pentostam

Treatment	Time of administration	
	Week 0*	Week 4**
Liposome-Pentostam 'intra lesion' .....	1	17
Free Pentostam 'intra lesion' .....	1	10
Liposome-Pentostam intravenous .....	6	3
Free Pentostam intravenous .....	17	15
Liposome-Pentostam subcutaneous .....	12	—
Free Pentostam subcutaneous .....	16	—
None .....	16	

All treated groups received a total of 250 mg/kg Pentostam (either free or liposome-entrapped). Treatment labelled 'subcutaneous' was administered at a site remote from the lesion.

\* Treatment given immediately after parasite inoculum.

\*\* Treatment delayed until palpable nodule observed.

the therapeutic efficacy of Pentostam. In the subcutaneous administration at the site of the infection ('intra-lesion'), however, both free Pentostam and liposome-encapsulated drug were equally effective at suppressing the development of the lesion, and were more effective than the intravenous route.

If treatment was delayed, however, until the formation of a palpable nodule, then the intravenous route of administration of liposomes was the most effective in inhibiting the development of the lesions. It is also apparent that the delayed intravenous administration of liposomes was more effective than dosing by the same route at the time of infection. Once a nodule had developed, the intra-lesion route of administration of either liposome-entrapped or free Pentostam was far less effective than when the treatment was given immediately following infection.

## Discussion

Cutaneous manifestations of leishmaniasis in man may be of long duration, may result in permanent tissue damage and in some cases even cause death. The disease is often refractory to treatment and when a cure is possible the drugs at present available are toxic and need to be given in large doses over a long period of time, leading to many undesirable side effects.

It is encouraging therefore that the efficacy of an antileishmanial drug in the treatment of experimental cutaneous leishmaniasis may be enhanced by entrapping it inside liposomes and that after the lesion has begun to develop treatment by the parenteral route is still effective.

There is a pressing need for improved drug delivery systems in the treatment of the forms of the disease where the infection is long standing, destructive (as in the case of mucocutaneous leishmaniasis), or widely disseminated as in diffuse cutaneous leishmaniasis, and following our observations of the efficacy of Pentostam entrapped within liposomes it may well be possible to apply liposome therapy in some of these cases, given the appropriate experimental models.

The mode of action of Pentostam against *Leishmania* is still unclear, but in the visceral form of the disease it is thought that the drug has direct toxicity on the parasite in macrophages and that this effect is enhanced by association with liposomes, which increase the concentration of the drug within the liver. In the treatment of cutaneous leishmaniasis by liposomes reported here it appears that this view of the mechanism of the drug activity is unsatisfactory, since although entrapment inside liposomes does increase the uptake of the drug by the cells of the reticulo-endothelial system, including those in the lesion, the introduction of liposomes into a cutaneous lesion is less effective in controlling a lesion, once it has developed, than is an intravenous dose of liposomes, although very much less drug reaches the lesion by the latter route. Whatever the mechanism of action of Pentostam administered within liposomes, this observation indicates

that it is interacting with tissues and parasites which are at sites other than the lesion. Further work is necessary to elucidate the mode of action of the drug combination.

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