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Non-ionic surfactant vesicles, niosomes, as a delivery system for the anti-leishmanial drug, sodium stibogluconate

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Liver and serum concentrations of antimony in the mouse have been determined after administration of sodium stibogluconate in the free, liposomal and niosomal form. High liver and low serum values were attained by the use of both vesicular formulations. Niosomal sodium stibogluconate was shown to be more active than free drug against experimental murine visceral leishmaniasis, an effect apparently dependent on maintaining high drug levels in the infected reticuloendothelial system.

Non-ionic surfactant vesicles or niosomes (Handjani-Vila et al 1979) appear to be similar in terms of their physical properties (Baillie et al 1985) to liposomes and have been shown (Azmin et al 1985) to modify the tissue distribution of entrapped, niosomal methotrexate. These surfactant vesicles may be regarded either as inexpensive alternatives, of non-biological origin, to liposomes, or perhaps in-vivo as a carrier system physically similar to the liposome, but with its own peculiar properties which can be exploited to attain different drug distribution and release characteristics.

Our aim has been to use drug carrier systems to enhance the chemotherapy of infection. In this paper we present our preliminary findings on the ability of niosomes to modify the tissue distribution and efficacy of sodium stibogluconate, an organic pentavalent antimony drug which is the drug of choice in the therapy of visceral leishmaniasis, a protozoan infection of the reticuloendothelial system (RES). The liposomal form of the drug is known to be much more effective than the free drug against animal models of the disease (for a review see Alving 1983).

MATERIALS AND METHODS

Materials

Sodium stibogluconate (Pentostam) equivalent to 0·28 to 0·32 mg Sb mg^{-1}, was obtained from The Wellcome Foundation, UK. Synthetic (>99% pure) L-α-phosphatidylcholine, dipalmitoyl (DPPC), dicetyl phosphate (approx. 97% pure by GC) and cholesterol, ash free, were used as received from Sigma. The single aliphatic chain non-ionic surfactant (Surfactant I, Baillie et al 1985) was obtained from L'Oreal, France. All other reagents were of analytical grade.

Methods

Male BALB/C mice of about 20 g were used and had free access to food.

Leishmania donovani (strain LV9, London School of Hygiene and Tropical Medicine) was maintained by passage through female Golden Syrian hamsters. A suspension of *L. donovani* (0·4 ml, 2×10^8 amastigotes) was administered intraperitoneally to the hamsters which were killed some 7 weeks after inoculation, the infected spleen being removed and homogenized in PSGEMKA buffer (Hart et al 1981). The released amastigotes were washed and resuspended in PSGEMKA buffer and used to infect further hamsters or to infect mice by tail vein inoculation. Mice were given 0·1 ml parasite suspension containing 2×10^7 *L. donovani* amastigotes.

Vesicle preparation. Niosomes were prepared by an ether injection method (Baillie et al 1985) in which 450 μmol of a surfactant–cholesterol mixture (7:3 molar ratio) dissolved in diethyl ether was injected slowly into 5 ml 300 mg ml^{-1} aqueous sodium stibogluconate solution at 60 °C. Liposomes were prepared by hydrating under a N_2 atmosphere 114 μmol of a mixture of DPPC, cholesterol and dicetyl phosphate (7:2:1 molar ratio) with 8 ml 300 mg ml^{-1} aqueous sodium stibogluconate solution at 45–50 °C for 2 h, with gentle agitation.

Untrapped drug was removed from niosome and liposome suspensions by dialysis (Visking tubing)

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against 300 mM glucose solution. Dialysed vesicle preparations were adjusted to the required drug concentration (on the basis of antimony content) by dilution with 300 mM glucose solution.

Antimony determination. Flame atomic absorptiometry (Pye Unicam SP90 Atomic absorptiometer) was used to determine the antimony content of vesicle preparations and tissue samples. The antimony standard was a solution of $SbCl_3$ in dilute HCl. The sensitivity of the method was 1 μg Sb ml^{-1}.

Vesicle preparations were disrupted in propanol before assay. Serum samples were diluted with water if necessary before assay. Liver samples were processed by the method of Heath et al (1984) and the resultant buffered extract diluted, if necessary, before assay.

Tissue distribution studies. Uninfected mice were injected via the tail vein with 0·2 ml of a 5 mg ml^{-1} sodium stibogluconate preparation. The preparations used were free drug as an aqueous solution and niosomal and liposomal suspensions of the drug. At 0·25, 1, 4 and 24 h post injection, mice were ether-anaesthetized, blood samples were removed by cardiac puncture and the liver was removed after killing the animal. At each sample time, the liver and serum samples from 5 animals were pooled.

Parasite clearance studies. Mice were infected with *L. donovani*, as described above, on day 1. With 5 mice per treatment group, mice were drug-treated on day 7 or on days 7 and 8 with 0·1 ml free or niosomal sodium stibogluconate. Daily dosages (mg drug via tail vein per mouse) were: one day treatment, 0·033, 0·3 free drug; 0·025, 0·25 niosomal drug, and two day treatment, 0·033 free drug, 0·025 niosomal drug. An infected untreated control group was also set up.

In all cases, mice were killed on day 14, the livers removed and duplicate Giemsa-stained impression smears prepared from each liver. The duplicate smears were examined microscopically (1000× magnification) and the mean number of parasites per 100 host liver cell nuclei counted.

RESULTS AND DISCUSSION

Antimony serum levels after free sodium stibogluconate administration were 21·0 μg ml^{-1} at 15 min, 23·3 μg ml^{-1} at 1 h, 1·9 μg ml^{-1} at 4 h and not detectable at 24 h. For both vesicular forms no serum antimony could be detected at any of these sampling times. These results together with those shown in Fig. 1 are typical of the rapid scavenging of most drug carrier systems from the systemic circulation by the RES. There is an indication in Fig. 1 that the liposomes were removed from the circulation more readily than the niosomes although further observations would be necessary to establish this.

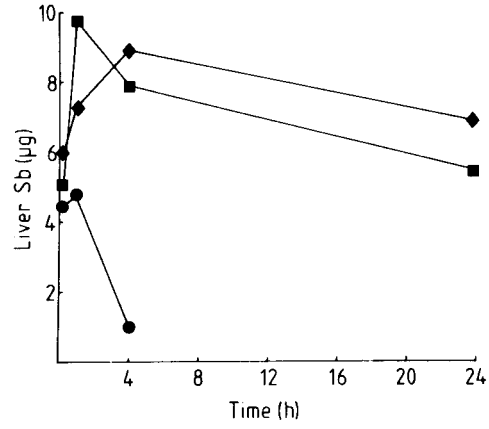

FIG. 1. Antimony levels in normal mouse liver at various times after dosing via the tail vein with 1 mg sodium stibogluconate in the form of (●) free solution or (■) liposomal or (◆) niosomal suspension. Each experimental point represents the value obtained by assaying the supernatant of the homogenate of livers pooled from 5 mice. No antimony was detected in livers at 24 h after dosing with free drug.

Both vesicular forms of the drug gave higher peak liver antimony levels, some two times higher, than the free form (Fig. 1). The greatest liver level of the metal was observed at 1 h after dosing for liposomal and free drug and at 4 h for the niosomal formulation. These peak liver levels represent for free, 1·5%; liposomal, 3·1% and niosomal, 2·8% of the total antimony administered. The value here for liposomes is in good agreement with that of Black & Watson (1977) of 3·43% at 1 h, although their values for free drug, e.g. 0·66% at 1 h, are lower than ours. The most pronounced effect of giving the drug in vesicular form, however, was the sustained high liver antimony levels up to 24 h, at least, after dosing; 4 h after administration of the free drug, liver antimony levels were down to 1 μg per organ, the sensitivity limit of the assay method used here. Extrapolation of the curves in Fig. 1 indicates that liver antimony levels were back to zero at about 5 h after dosing for free drug and after not less than 70 h for the vesicular forms. The decay in liver antimony levels for the niosomal and liposomal formulations were similar, suggesting a similar route of excretion for the vesicular metal from this organ. This was somewhat disappointing since it was hoped that in the liver the

niosomes would be less easily degraded than the liposomes and so would provide a more sustained effect. We suggest that the similar rate of loss of antimony from the liver (Fig. 1) for liposomes and niosomes indicates that, in this case, simple diffusion of drug from both liposome- and niosome-loaded liver was the rate-limiting process and that active processes such as lysosomal degradation of vesicles were not critical. The data for the vesicular material, compared with that for free drug, are in accord with a liver depot, presumably in the form of phagocytosed vesicles, which hinders release from this tissue.

Parasite burden. The effects of free and niosomal drug on the liver amastigote burden are shown in Fig. 2. At the doses used, both formulations significantly reduced the number of *Leishmania* parasites in the liver. On the basis of an equi-effective dose of sodium stibogluconate, it is apparent that, under the conditions used here, niosomal drug was more than an order of magnitude more active than the free form. We have not determined the efficacy of liposomal sodium stibogluconate in our system, but

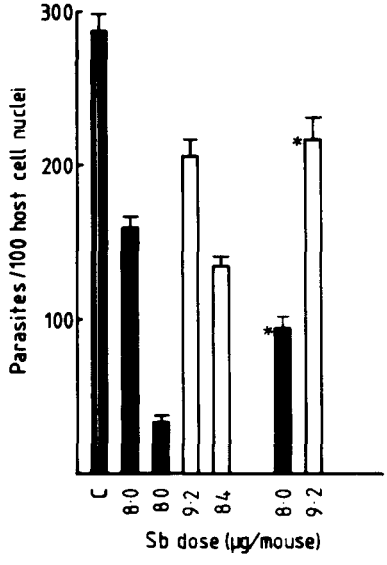

FIG. 2. Histogram of the number of *L. donovani* amastigotes per 100 liver cell nuclei obtained by counting Giemsa-stained liver impression smears. Mice were infected with 2×10^7 amastigotes on day 1, dosed with niosomal (■) or free (□) sodium stibogluconate on day 7 or on days 7 and day 8 (*) and the parasite burden determined on day 14. The infected control group, C, was not treated. Parasite burden values are the mean for 5 mice, each liver counted in duplicate. Bars indicate standard error of mean. There was no significant difference (Mann-Whitney U-test, $P = 0{\cdot}05$) between parasite burdens after dosing with 0·025 mg niosomal drug (8 μg Sb) and 0·3 mg free drug (84 μg Sb).

the reported efficacy increase of this form of the drug over the free form is 200× (Black & Watson 1977) and 700× (Alving et al 1978a), and a similar increase reported for other antimonials such as meglumine antimonate (Alving et al 1978b) and potassium antimony tartrate (New et al 1978). These results appear to indicate a superiority of the liposome carrier over the niosome type in animal models of the disease. However the % parasite suppression found (Fig. 3) with niosomal sodium stibogluconate is in good agreement with the data of Black & Watson (1977) and Alving et al (1978a) for liposomal drug. The discrepancy between their results and ours for free drug may well be explained by the use of different host animals (species and strain) and different dosage schedules.

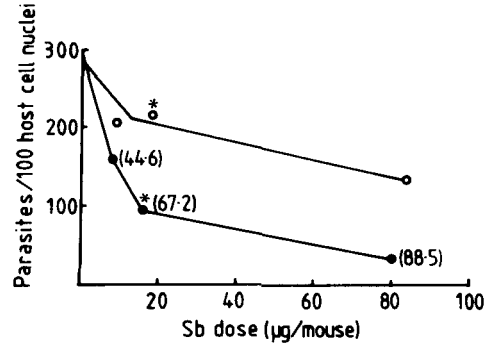

FIG. 3. The data of Fig. 2 plotted to give response curves for free (○) and niosomal (●) sodium stibogluconate. The points marked (*) are plotted as the total antimony dose given over 2 days in the form of niosomal, 16 μg Sb, and free, 18·4 μg Sb, drug per mouse. The two doses of niosomal drug appeared to be additive. The values in parentheses show the percentage suppression of parasite burden obtained by niosomal drug.

Significantly, the effects of two doses of niosomal sodium stibogluconate given on successive days were additive (Fig. 3) which may be explained on the basis of the liver depot effect seen (Fig. 1) with this form of the drug. No additive effect was apparent with the free drug and the effects of the single and double doses of 0·033 mg were not significantly different. In a separate experiment, empty niosomes were found not to affect liver parasite burdens.

It would appear then that niosomes and liposomes are similar in terms of sodium stibogluconate distribution in the mouse liver and serum, and that the chemical difference between these carrier systems, non-ionic surfactant and phospholipid, are relatively unimportant in this respect. However, other (non-target) tissue such as kidney or heart has not yet been assayed. In addition, niosomal sodium stiboglu-

conate, like the liposomal drug, is more effective than the free drug and the marked liver loading observed with the vesicular drug is probably adequate to explain the enhanced anti-leishmanial activity.

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REFERENCES

Alving, C. R. (1983) Pharmacol. Ther. 22: 407–424

Alving, C. R., Steck, E. A., Chapman, W. L. Jr., Waits, V. B. Hendricks, L. D., Swartz, B. M. Jr., Hanson, W. L. (1978a) Proc. Natl. Acad. Sci. USA 75: 2959–2963

Alving, C. R., Steck, E. A., Hanson, W. L., Loizeaux, P. S., Chapman, W. L. Jr., Waits, V. B. (1978b) Life Sci. 22: 1021–1026

Azmin, M. N., Florence, A. T., Handjani-vila, R. M., Stuart, J. F. B., Vanlerberghe, G., Whittaker, J. S. (1985) J. Pharm. Pharmacol. 37: 237–242

Baillie, A. J., Florence, A. T., Hume, L. R., Muirhead, G. T., Rogerson, A. (1985) Ibid. 37: 863–868

Black, C. D. V., Watson, G. J. (1977) Trans. R. Soc. Trop. Med. Hyg. 71: 550–552

Handjani-Vila, M., Ribier, A., Rondot, B., Vanlerberghe, G. (1979) Int. J. Cos. Sci. 1: 303–314

Hart, D. T., Vickerman, K., Coombs, G. H. (1981) Parasitology 82: 345–355

Heath, S., Chance, M. L., New, R. R. C. (1984) Mol. Biochem. Parasitol. 12: 49–60

New, R. R. C., Chance, M. L., Thomas, S. C., Peters, W. (1978) Nature 272: 55–56