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Visceral leishmaniasis in the BALB/c mouse: sodium stibogluconate treatment during acute and chronic stages of infection: II. Changes in tissue drug distribution

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Summary

Animals with either an acute or chronic *Leishmania donovani* infection were treated with free or vesicular sodium stibogluconate (SSG) and tissue antimony levels and parasite suppressions in spleen, liver and bone marrow (femur) sites assessed. Treatment with free or vesicular SSG caused a significant suppression in spleen ($p < 0.01$) and liver ($p < 0.001$) parasite numbers compared to control values in the acute model. In the chronic model, treatment with free SSG was ineffective against spleen, liver and bone marrow parasites (similar burdens present in control and free drug treated animals) and vesicular SSG only significantly suppressed liver ($p < 0.005$) parasite burdens compared with controls. Dosing with free SSG resulted in higher mean tissue antimony concentrations in the liver, spleen, femur and kidney of acute infection mice compared to those obtained in chronic infection animals, however these differences were only statistically significant in the spleen ($p < 0.05$). If the marked hepatosplenomegaly caused by *L. donovani* infection is taken into account then the total amount of antimony present in the liver and spleen after free drug dosing was similar in acute and chronic infection mice. Administration of vesicular SSG to the two groups of mice resulted in higher antimony concentrations and higher total antimony levels in the spleen and liver compared with those achieved by free drug treatment ($p < 0.05$). The implications of the results are discussed.

Introduction

We have shown in a murine model of visceral leishmaniasis (VL) that the efficacy of sodium stibogluconate treatment decreased as the chronicity of the infection increased (Baillie et al., 1989). However, treatment with carrier forms of the drug was less sensitive to the influence of delayed drug treatment. On the basis of those and other findings (Carter et al., 1988, 1989a,b) we proposed (Baillie et al., 1989) that the outcome of drug therapy of experimental murine VL depended on three factors: the innate activity of the drug, host factors (including immunological

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status) and pharmacological factors. In this paper, we present data to suggest that there is a difference in the pharmacokinetics of stibogluconate between acute and chronic infection mice which is manifest as a difference in tissue antimony distribution between acute and chronically *Leishmania donovani*-infected mice, after treatment with the free or vesicular forms of sodium stibogluconate.

Most chemotherapeutic studies of experimental VL have been based on the use of acute infection models to test the efficacy of drugs (Gellhorn and Van Dyke, 1946; Berman et al., 1988) and novel carrier formulations of drugs (Black et al., 1977; New et al., 1981; Baillie et al., 1986). In such studies, infected animals are treated after a relatively short period and the effects of the therapies assessed by comparing parasite burdens in various organs, such as the liver or spleen, of treated and control animals. However, in the clinic, patients usually present when the disease has progressed to a chronic phase of infection (Bryceson, 1987) and in that situation chemotherapy has to contend with the influence of the stage of infection on therapeutic outcome, a problem not addressed in the typical acute animal models (Alving, 1983). Apart from the immunosuppressive aspects of *L. donovani* infection, the causal agent of VL, several pathological changes are induced in the host (reviewed by Ridley, 1987) which may influence drug distribution and other pharmacokinetic parameters. A marked hepatosplenomegaly (Cole, 1944) is the most obvious pathognomonic sign which has implications for blood and hence drug distribution, although other parasite-induced changes also occur, such as pancytopenia (Cartwright et al., 1948), the appearance of multiple intralobular granulomas in the liver (Naik et al., 1976; Gonzalez et al., 1988) and in severe cases, haemosiderotic nodules in the spleen (Sen Gupta and Chatterji, 1960).

Materials and Methods

Materials

Sodium stibogluconate (SSG, Pentostam®), equivalent to 0.32 mg pentavalent antimony (Sb^V) mg^{-1}, was obtained from the Wellcome Foundation, U.K. Throughout, drug concentrations and dosages are expressed in terms of Sb^V. The single chain non-ionic surfactant (Surfactant IV, Stafford et al., 1988) was obtained from L'Oreal, France. Ash-free cholesterol (CHOL), dicetyl phosphate (DCP) and synthetic L-α-dipalmitoylphosphatidylcholine (DPPC >99% pure) were obtained from Sigma. These materials were used as received and all other reagents were, unless stated, of analytical grade.

Vesicle preparation

Drug-loaded liposomes were prepared from DPPC and CHOL on a 7:3 molar basis as described by Collins et al. (1990a) and drug-loaded non-ionic surfactant melt vesicles were prepared from Surfactant IV, CHOL and DCP on a 5:4:1 molar basis using methods described by Collins et al. (1990b). The vesicles were sized (Baillie et al., 1989) and had a mean hydrodynamic diameter of 110.5 nm, and polydispersity factor of 0.13–0.22 for liposomes, the corresponding values being 149.8 nm and 0.16–0.23 for non-ionic surfactant vesicles. From the z average diffusion coefficient (Koppel, 1972) the diameter is weighted towards the larger vesicles in the sample.

Animals

8–10-week-old male and female inhouse inbred BALB/c mice were used throughout experiments. Inhouse bred Golden Syrian hamsters (*Mesocricetus auratus*) were used to maintain the parasite using the method described by Carter et al. (1987).

Experimental design

Unanaesthetised mice were infected via the tail vein with $1-2 \times 10^7$ amastigotes in 0.2 ml RPMI 1640 (Gibco) of *L. donovani* (LV9) harvested from the spleen of a freshly killed hamster. Groups ($n \geq 4$) of unanaesthetised infected animals were treated on day 7 (acute infection) or day 78 (chronic infection) with three doses of 0.2 ml of one of the following: saline (controls); SSG solution (equivalent to a total dose of 133.2 mg Sb^V/kg); or SSG-loaded vesicles (equivalent to a total dose of 13.3 mg Sb^V/kg). There was an

interval of 1.5 h between drug doses. One group of animals from each treatment was killed 1 h after the last drug dose and tissue antimony in the spleen, liver, heart and left femur determined using the methods previously described by Collins et al. (1992). A second group of mice from each treatment were killed 6 days after their last drug dose and the number of parasites per 1000 host cell nuclei in the liver, spleen and bone marrow assessed as described by Carter et al. (1987). The number of Leishman Donovan units (LDU) was calculated per organ for the liver and spleen using the formula: LDU = number of amastigotes per 1000 host cell nuclei × the organ weight (g, Bradley and Kirkley, 1977).

In a separate experiment, uninfected unanaesthetised mice were treated with 0.2 ml of SSG solution equivalent to 44.4, 333 or 666 mg Sb^v/kg. Animals were killed 0.5 h post-dosing and the liver, spleen and femurs removed for tissue antimony analysis.

Presentation and statistical analysis of data

Parasite burdens of the spleen and liver are expressed as mean $\log_{10}$ LDU/organ + standard error (SE), whereas the bone marrow counts are expressed as mean $\log_{10}$ number of parasites/1000 host cell nuclei + SE. The antimony concentration in each tissue is shown as the mean antimony concentration + SE (μg/g dry weight). Log_{10} transformed parasite data were analysed using a one-way analysis of variance or an independent 't'-test. Tissue antimony data were analysed using the non-parametric Mann-Whitney test.

Results

Treatment of mice with an acute *L. donovani* infection with SSG solution ('free' SSG) or vesicular SSG significantly suppressed spleen ($p < 0.01$) and liver parasite ($p < 0.001$) numbers, but failed to reduce bone marrow parasite burdens compared with control values (Fig. 1a). Vesicular drug dosing resulted in a greater reduction in spleen ($p < 0.05$) and liver ($p < 0.025$) parasite burdens compared with that achieved after free drug dosing. Administration of either SSG formulation to chronic infection mice showed that the antiparasitic effects of the drug were influenced by the stage of infection (Fig. 1b). Free SSG treatment had no significant effect on spleen ($p > 0.25$) or liver parasite ($p > 0.10$) burdens and in the case of vesicular SSG, although there was significant reduction in liver parasite numbers compared with controls ($p < 0.005$), spleen parasite burdens were not affected. Neither treatment significantly reduced bone marrow parasite burdens of chronic infection mice compared with controls ($p > 0.10$). Comparison of the antiparasitic effect of vesicular SSG in the liver in the two

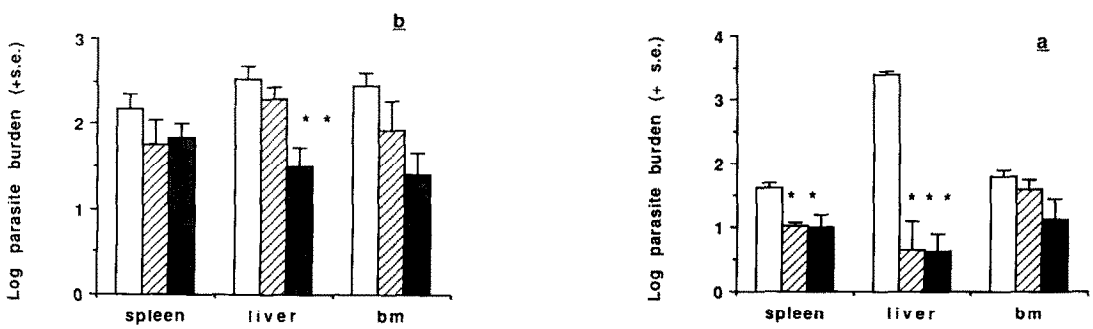

Fig. 1. Mean parasite burdens (+SE, $n \geq 4$) for spleen, liver and bone marrow from mice treated intravenously (tail vein) with: saline, control mice (□); sodium stibogluconate (SSG) solution (▨, total Sb dose 133.2 mg kg^{-1}); vesicular SSG (■, total Sb dose 13.3 mg kg^{-1}). BALB/c mice were infected with *L. donovani* amastigotes (day 0), treated on day 7 (acute infection, a) or day 78 (chronic infection, b) and assessed for parasite burdens 6 days after treatment. Asterisks indicate parasite burdens significantly different from the relevant control values; ** $p < 0.01$, *** $p < 0.001$.

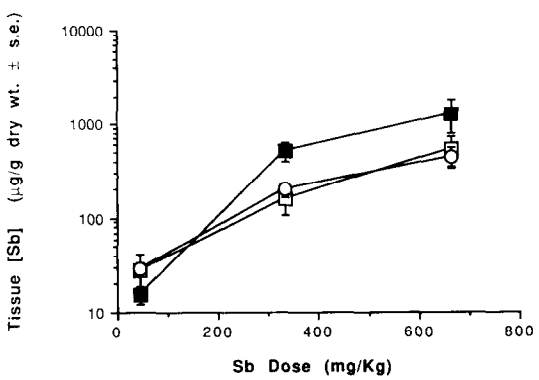

Fig. 2. Relationship between antimony dose and mean tissue antimony concentration (±SE, $n = 5$) in liver (□), spleen (■) and femur (○) of uninfected BALB/c mice at 0.5 h after administration of a single intravenous (tail vein) 0.2 ml dose of SSG solution.

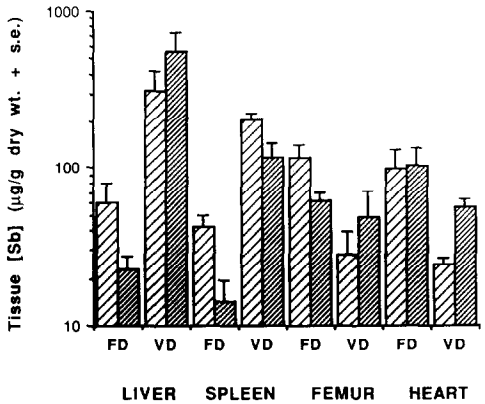

Fig. 3. Mean tissue antimony concentrations (+SE, $n = 5$) for liver, spleen, heart and femur from acute and chronic infection mice after the administration (tail vein) of sodium stibogluconate (SSG) solution (FD, total dose 133.2 mg Sb kg^{-1}) or vesicular SSG (VD, total dose 13.3 mg Sb kg^{-1}). BALB/c mice were infected with *L. donovani* amastigotes (day 0), treated on day 7 (acute infection, ▨) or day 78 (chronic infection, ▨) and the tissues assayed for antimony content 1 h after treatment.

stages of the infection showed that a greater decrease of parasite numbers was obtained in the acute model (acute: 96 ± 2%, $n = 4$; chronic: 90 ± 4%, $n = 4$).

Fig. 2 shows the relationship between SSG dose and tissue antimony concentration achieved in the liver, spleen and femur of uninfected mice. Antimony concentrations in the liver and spleen of SSG-treated acute infection mice (Table 1) were of the same order as those determined by interpolation from the tissue concentration/dose curve. However, in SSG-treated chronic infection mice, liver and spleen antimony concentrations (Table 1) were significantly lower than would be predicted from Fig. 2. Similar antimony concentrations were present in the femurs of SSG treated uninfected and chronic infection mice whereas those in acutely infected animals were higher.

Treatment of acute or chronic infection mice with vesicular SSG gave higher concentrations of antimony in the spleen and liver than free drug treatment ($p < 0.05$) but not in the femur or heart ($p > 0.05$, Fig. 3). The vesicle-mediated increase in tissue antimony concentration, when present, was more marked in the chronic model. For example, in the acute model, treatment with vesicular SSG resulted in 5-fold greater liver and spleen antimony concentrations than those obtained after free SSG treatment whereas in the

TABLE 1

Mean tissue antimony concentrations (μg/g dry weight ±SE, $n = 4$) in the liver, spleen and femur of uninfected, acute and chronic infection mice, for a fixed dose of 0.2 ml of SSG solution (equivalent to 133.2 mg/kg Sb^V) given via the tail vein

Infection status	Tissue antimony concentration (μg/g dry wt)		
	Liver	Spleen	Femur
Uninfected	50.0 ± 16.0 (1.17 ± 0.07)	45.0 ± 10.0 (0.24 ± 0.01)	53.0 ± 9.0
Acute	60.4 ± 19.7 (1.31 ± 0.01)	42.7 ± 7.4 (0.3 ± 0.01)	116.2 ± 23.4
Chronic	22.9 ± 4.9 (2.28 ± 0.05)	14.2 ± 5.1 (1.52 ± 0.04)	62.3 ± 7.3

Values in parentheses are the mean liver and spleen weights for the various groups of animals (g ± SE, uninfected, $n = 10$; acute and chronic, $n = 4$). For uninfected mice, the tissue concentration data were obtained by interpolation from the curves in Fig. 2; values for acute and chronic infection mice are also plotted in Fig. 3.

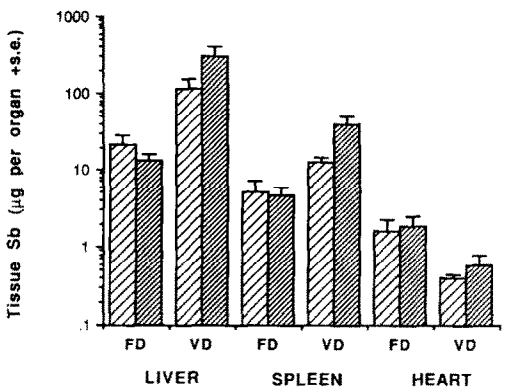

Fig. 4. Mean organ antimony content (+SE, $n = 5$) for liver, spleen and heart from acute and chronic infection mice. Legend as in Fig. 3.

chronic model, 24-fold (liver) and 8-fold (spleen) increases in antimony concentration were obtained. If, however, organ weights (Table 1), quite different in the two stages of infection, are used to calculate the total amount of antimony present in each organ (Fig. 4), then it is found that similar total amounts of antimony were present in the liver, spleen and the heart of acute and chronic infection animals ($p > 0.05$) given free SSG. Treatment with vesicular stibogluconate resulted in similar total heart antimony levels in both groups of mice and higher total antimony levels in the liver and spleen of chronic infection mice ($p < 0.05$).

Discussion

The data confirm our previous findings (Baillie et al., 1989) that in this mouse model of VL the antiparasitic activity of SSG is dependent on the stage of infection and that the vesicular formulation (intrinsically more active than the free drug) is less sensitive to this effect. We proposed (Baillie et al., 1989) that the outcome of the therapy of experimental VL was the sum of the innate antiparasitic activity of the drug, host factors and the pharmacokinetics of the drug. We now propose that the differences in susceptibility of liver and spleen parasites to free and vesicular SSG between acute and chronic models of infection can be explained on the basis of altered pharmacokinetics.

In common with the chemotherapy of all types of infection, it is reasonable to expect that the critical parameter which defines drug activity is the drug concentration achieved at site of infection. Therefore, tissue antimony concentrations might be expected to give an indication of the antileishmanial activity of SSG at a particular site. Thus, in the case of free drug, since treatment of chronic infection mice resulted in lower spleen and liver antimony concentrations compared with those achieved in acute infection animals, then the lower antileishmanial activity of SSG in these sites in the chronic model would not be unexpected. The hepatosplenomegaly of VL, which was more marked in the chronic infection, can adequately account for the lower tissue antimony concentrations, since the same total amount of antimony was present in the livers and spleens of both groups of animals.

The greater efficacy of vesicular SSG compared with free SSG in both the acute and chronic models against liver and spleen parasites was in accord with the higher tissue antimony concentrations achieved by dosing with this novel drug formulation. Even taking into account the difference in organ weights for the two stages of the infection, vesicular SSG treatment resulted in higher total antimony levels in the spleen and liver of chronic infection mice. The marked hepatosplenomegaly of the chronic infection appeared to promote vesicular SSG uptake perhaps as a secondary consequence of a disease associated inflammatory process.

It has been suggested that it should be possible to increase the efficacy of (free) SSG against VL in man by increasing the drug dose administered (Bryceson, 1987). Our results indirectly support this suggestion since in the chronic infection, which is the type of VL treated in the clinic, liver and spleen antimony concentrations were depressed, implying a smaller concentration gradient for drug permeation of infected cells and thus impaired parasite killing. However, in the heart similar antimony concentrations were found in the acute and chronic infection mice, suggesting that heart antimony concentrations are a function

of systemic concentrations. Thus, increasing the SSG dose to compensate for the dilution effects of the hepatosplenomegaly would simply increase heart antimony loading and the risk of cardiotoxicity, a factor which limits clinical use of high dose antimonial drugs (Bryceson, 1987). In contrast, treatment with vesicular drug promoted liver and spleen loading and minimised the heart's exposure to antimony, since it is a non-phagocytic tissue.

In conclusion, vesicular delivery of SSG effectively decouples tissue from systemic concentrations and thus provides a low risk means of achieving high target tissue concentrations without increasing the dose.

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