*International Journal of Pharmaceutics*, 53 (1989) 129–137
Elsevier



IJP 01794

# A direct comparison of sodium stibogluconate treatment in two animal models of human visceral leishmaniasis, mouse and hamster

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(Received 30 November 1988)
(Modified version received 6 January 1989)
(Accepted 12 January 1989)

*Key words*: *Leishmania donovani*; Sodium stibogluconate; Liposome; Acute infection; Chronic infection; BALB/c mouse; B10 mouse; Golden syrian hamster

## Summary

The effect of sodium stibogluconate therapy, in the free or liposomal form, on the spleen, liver and bone marrow parasite burdens of *L. donovani* infected mice (BALB/c and B10) and hamsters was studied. Animals were infected i.v. (tail vein in mice, jugular vein in hamsters) and treated on either days 7 and 8 postinfection (acute model) or on days 31 and 32 postinfection or later (chronic model) with free (44.4 mg Sb (v)/kg/day) or liposomal (7.1 mg Sb (v)/kg/day) drug. Six days after the second drug dose animals were killed and parasite burdens assessed. Comparison of the parasite burdens in untreated *L. donovani* infected mice (B10 and BALB/c) and hamsters showed that higher burdens were obtained in hamsters in all 3 infection sites when the data were expressed as Leishman-Donovan units. However, if the data were expressed as the number of parasites/1000 host cell nuclei, liver burdens were similar in the two species although the parasite burdens remained higher in the spleen and bone marrow of hamsters. In the acute model, both free and liposomal drug treatment significantly reduced liver and spleen parasite burdens in BALB/c mice compared with controls, the liposomal form being significantly more suppressive at a sixth of the free drug dose. The two treatments had little effect on murine bone marrow burdens. In the hamster, the relative efficacies of the free and liposomal forms of the drug in the liver were similar to that in the mouse but in the spleen and bone marrow both treatments markedly reduced parasite numbers. The parasites in the hamster were therefore much more susceptible to drug treatment and this species-dependent effect was most marked in the bone marrow. In mice suffering from the chronic infection the effectiveness of free and liposomal stibogluconate decreased with the length of infection. A similar effect was also demonstrated in hamsters.

## Introduction

Sodium stibogluconate, the mainstay in the therapy of human visceral leishmaniasis caused by *Leishmania donovani* is, as a highly water-soluble compound, rapidly excreted. As a consequence, the drug has a short half-life, which necessitates daily administration over a period of twenty or more days to achieve a clinically useful parasite suppression. In spite of the relatively recent trend to use higher doses, a proportion of individuals still relapse after sodium stibogluconate therapy (WHO, 1984). Results from chemotherapy experiments in a mouse model of visceral leishmaniasis

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suggest that treatment failure may be due to the difficulty in completely clearing parasites from some sites of infection such as the bone marrow. The surviving parasite population at such sites may subsequently recolonise the host (Carter et al., 1988).

The apparent inaccessibility of the parasites in such 'deep' tissue sites may be regarded as a problem of drug targeting and in this context the use of stibogluconate and other drugs in liposomal form which allows modification of drug distribution and excretion patterns would seem to be a promising approach. The apparent resistance of 'deep' site parasites to stibogluconate may also have a host genetic basis, since in the mouse model we have shown that both major histocompatibility (MHC) genes and non-MHC genes can effect the outcome of drug therapy (Carter et al., 1989a). By selection of appropriate mouse strains it is possible to obtain either of two extremes with regard to the outcome of stibogluconate therapy; a significant reduction in parasite numbers in the liver, spleen and bone marrow or parasite suppression in the liver only. This strain effect in the mouse was apparent with stibogluconate in the free and vesicular, liposomal and niosomal, forms.

Various hamster models have been used to investigate the chemotherapy of visceral leishmaniasis and for the assessment of liposome-mediated therapy in particular, Alving et al. (Alving et al.., 1978a, b; 1980; 1984, Alving 1986; Berman et al., 1986; Hanson et al., 1977) have made extensive use of this species. Where the mouse has been used as the host species there have been strain differences (Berman et al., 1986; Carter et al., 1988) and different experimental protocols which make comparison of the results of different workers difficult. An additional complication is that in most studies, the efficacy of drug treatment is based on changes in parasite numbers in only one site, for example, the spleen (Goodwin et al., 1944, 1945) or the liver (Alving et al., 1984; Adinolfi et al., 1985), which also invalidates comparisons between treatments.

In order to evaluate the efficacy of free and liposomal stibogluconate therapies in the hamster we have used the standard protocol we have developed in the mouse model, which involves assessment of parasite burdens in the spleen, liver and bone marrow. This is to our knowledge the first study which directly compares, in three distinct sites of infection, the hamster and mouse as hosts for *Leishmania donovani* both in terms of their susceptibility to infection, and the outcome of free and liposomal stibogluconate therapy.

## Materials and Methods

*Materials*

Sodium stibogluconate (Pentostam) equivalent to 0.32 mg Sb(v)/mg was obtained from the Wellcome Foundation, U.K. Synthetic (> 99% pure) L-α-phosphatidylcholine (DPPC) and ash-free cholesterol (CHOL) were obtained from Sigma. Benzalkonium chloride was obtained from Koch Light Laboratories Ltd. Materials were used as received and all other reagents were of analytical grade.

Liposomes comprised 70% amphiphile (DPPC) and 30% CHOL, on a molar basis, and were prepared using procedures already described (Baillie et al., 1986). Briefly multilamellar liposomes were produced by dissolving 150 μmol of DPPC/CHOL mixture in 10 ml chloroform in a 50 ml round-bottomed flask. The solvent was removed at room temperature (20 °C), under reduced pressure and the resulting film hydrated with 5 ml drug solution at 50–60 °C with gentle agitation. Sonicated liposomes were produced by probe sonicating the multilamellar preparation at 60 °C for 3 min using an M.S.E. 150 W sonicator, fitted with a titanium probe, set at approximately 10–15% of maximum power output.

The sonicated vesicular suspension was sized by photon correlation spectroscopy at a 90° scattering angle using a Malvern Instruments Type 7027 60 channel correlator in conjunction with a He/Ne laser (Siemens), wavelength 632.8 nm, nominal power output 40 mW. The mean hydrodynamic diameters of the sonicated vesicles used in these studies were found to be about 100–120 nm, polydispersity factor, 0.25–0.30. Derived from the $z$ average diffusion coefficient (Koppel, 1972), these hydrodynamic diameters are weighted towards the larger vesicles in the sample so that a

large proportion of the vesicles will have diameters of $< 100$ nm.

*Animals*

Female inbred BALB/c mice (8–10 weeks old, wt. 20–25 g), male and female bred Golden syrian hamsters (wt. 100–120 g, *Mesocricetus auratus*) bred in our laboratories and inbred C57BL/10ScSn, hereafter referred to as B10 mice (wt. 19–23 g) obtained from Harlan Olac, Ltd., Shaw's Farm, Blackthorn, Bicester, U.K., were used throughout experiments. Hamsters were also used to maintain the parasite.

*Parasite*

*Leishmania donovani* (LV9) was maintained as described by Carter et al. (1988). Both species of animals were infected by i.v. injection (mice, tail vein without anaesthetic; hamsters, jugular vein with anaesthetic) of $1-3 \times 10^7$ amastigotes in 0.2 ml. Hamsters were anaesthetised by an i.p. injection of pentobarbitone sodium (Sagatal, May and Baker Ltd., Dagenham, 7.8–9 mg/hamster) and the ventral neck surface washed with 0.1% benzalkonium chloride solution. A midline incision into the neck was made and the fatty tissue was teased apart to expose the oesophagus. The left jugular vein was located and parasites were injected using a 25-gauge needle, whilst the vein was immobilised using two pairs of forceps. The incision was closed with 7.5 mm Michelle clips (Arnold Horwell, London, U.K.) and swabbed with antiseptic.

*Parasite distribution*

The method of determining parasite burdens (number/1000 host cell nuclei) in liver, spleen and bone marrow has been described by Carter et al. (1988). 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 $\times$ the organ weight (g) (Bradley and Kirkley, 1977).

*Parasite suppression*

Infected animals were dosed (mice, 0.2 ml via the tail vein without anaesthetic and hamsters, 0.4 ml via the jugular vein with anaesthesia on two consecutive days postinfection with one of the following: distilled water (controls); sodium stibogluconate solution (equivalent to 44.4 mg Sb (v)/kg); or a suspension of liposomal drug (equivalent to 7.1 mg Sb (v)/kg). In the hamsters, alternate jugular veins (right, day 1; left, day 2) were used, by the method described for infecting the animals, the Michelle clips being removed prior to injection. Six days after the second injection parasite numbers in the spleen, liver and bone marrow of controls and drug-treated animals were determined. Throughout, the day postinfection on which the animals were killed, day 14, 38, 56, 58 or 84, is given (also referred to as the length of infection), drug treatment occurring 7 and 6 days prior to this time. Mice were killed by cervical dislocation and hamsters by ether anaesthesia.

*Presentation and statistical analysis of data*

Parasite burdens of the spleen and liver are expressed as mean LDU/organ $\pm$ S.E.M. and the bone marrow burden as mean number of parasites/1000 host cell nuclei $\pm$ S.E.M. The effect of sodium stibogluconate therapy on parasite burdens in each tissue site is also expressed as the mean % parasite suppression in experimentals compared with controls. This was calculated by obtaining the overall group mean value of the decrease in parasite burden for each individual experimental animal (based on LDU values in the spleen and liver, and as the mean number of amastigotes/1000 host cell nuclei in the bone marrow) relative to the mean parasite burden for that site in the appropriate control group. Parasite data were analysed using an independent *t*-test or a one way analysis of variance on the $\log_{10}$ transformed data and parasite suppression by the non-parametric Mann–Whitney test.

## Results

In both species there was a difference between the spleen and liver weights of untreated, infected animals (Table 1) and those of uninfected control animals because of the hepatosplenomegaly resulting from visceral leishmaniasis. There was also an inter-species difference in organ weights which

TABLE 1

*L. donovani parasite burdens in the spleen, liver and bone marrow of mice (BALB/c and B10) and hamsters on day 14 postinfection*

| | Spleen | Liver | Bone marrow |
|---|---|---|---|
| BALB/c | | | |
| N | $0.48 \pm 0.06$ | $2.17 \pm 0.25$ | $0.51 \pm 0.03$ |
| LDU | $0.17 \pm 0.02$ | $2.71 \pm 0.30$ | |
| organ wt (I) | $0.35 \pm 0.02$ | $1.25 \pm 0.05$ | b. wt. = $19.31 \pm 0.64$ |
| organ wt (U) | $0.17 \pm 0.01$ | $0.91 \pm 0.05$ | b. wt. = $21.16 \pm 0.81$ |
| $\Sigma P$ | | $6.77 \times 10^{11}$ | |
| B10 | | | |
| N | $0.28 \pm 0.06$ | $2.06 \pm 0.30$ | $0.63 \pm 0.02$ |
| LDU | $0.07 \pm 0.02$ | $2.96 \pm 0.47$ | |
| organ wt. (I) | $0.26 \pm 0.04$ | $1.44 \pm 0.07$ | b. wt. = $21.66 \pm 0.40$ |
| organ wt. (U) | n.d. | n.d. | b. wt. = n.d. |
| $\Sigma P$ | | $7.31 \times 10^{11}$ | |
| Hamster | | | |
| N | $7.53 \pm 1.72$ | $2.89 \pm 0.32$ | $1.62 \pm 0.29$ |
| LDU | $10.01 \pm 2.90$ | $22.95 \pm 3.54$ | |
| organ wt. (I) | $1.51 \pm 0.23$ | $7.89 \pm 0.93$ | b. wt. = $100.57 \pm 5.35$ |
| organ wt. (U) * | 0.25 | 10.76 | b. wt. = 206.00 |
| $\Sigma P$ | | $6.92 \times 10^{12}$ | |

Animals were infected with $1.6 \times 10^7$ *L. donovani* amastigotes in 0.2 ml administered via the tail (mouse) and jugular (hamster) vein. Parasite burdens ($\times 10^{-3}$) are shown as the no. per 1000 host cell nuclei ($N$) and, for spleen and liver, also as LDU = N × organ wt. The animal weight is shown along with a calculated total, whole body, parasite burden, $\Sigma P = (LDU_{spleen} + LDU_{liver} + N_{bone\ marrow}) \times 2 \times 10^8$ (after Stauber, 1958). Spleen, liver and body weights of infected (I) and uninfected (U) animals are shown. Throughout weights are in g and all values shown are means ± S.E.M. ($n = 5$). n.d., not done. * $n = 1$.

reflects amongst other things the obvious body weight difference between the species. The disease induced increases in organ weight (body weight corrected, infected organ weight/infected body weight : uninfected organ weight/uninfected body weight) were: spleen, BALB/c mouse, ×2.25; hamster, ×12.5 and liver, BALB/c mouse, ×1.5; hamster, ×1.5. As a consequence of organ weight differences the data have been presented in two ways (Table 1). Firstly, organ weights were ignored and the species compared on the basis of parasite numbers per 1000 host cell nuclei. No species difference in hepatic parasite burdens was observed but greater numbers were found in the spleen and bone marrow of hamsters. Secondly, on an LDU basis, however, there were 2–10 fold greater burdens in the liver and spleen of hamsters compared with those sites in the mouse (Table 1, Fig. 1). Calculation of the total parasite burden ($\Sigma P$, Table 1) showed this to be 10 × higher in the hamster than in the mouse although there was only a 5 fold difference in body weight.

In summary it was found that over an inoculum range (Fig. 1), which spanned that used routinely to infect animals in drug studies, the parasite burdens at all 3 infection sites (bone marrow data not shown) in the hamster and mouse had attained a plateau value which was higher in the hamster than the mouse, except when liver burdens were expressed as numbers per 1000 host cell nuclei (Table 2).

Assessment of the outcome of stibogluconate therapy on day 14 postinfection showed that in BALB/c mice, free drug (total dose 88.8 mg Sb (v)/kg) significantly reduced both liver ($P < 0.001$) and spleen ($P < 0.025$) parasite burdens compared with controls (Fig. 2), although bone marrow burdens were similar to control values. Treatment with liposomal stibogluconate (total dose 14.2 mg Sb (v)/kg) significantly reduced parasite numbers in the liver ($P < 0.001$) and the spleen ($P < 0.025$) but had no significant effect on bone marrow parasites compared with controls (Fig. 2). Against liver parasites in the mouse, the liposomal therapy

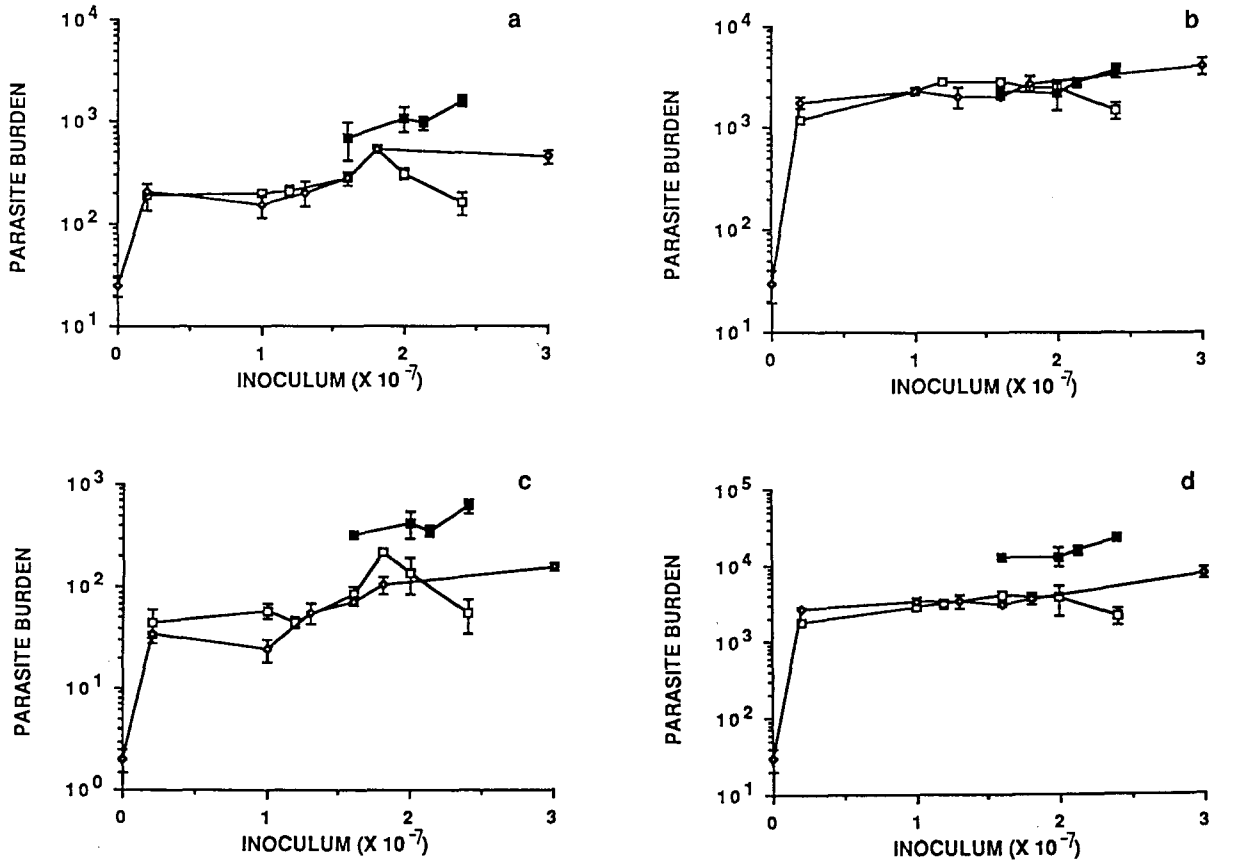


Fig. 1. Parasite burdens on day 14 postinfection in the spleen (a and c) and liver (b and d) of mice (B10 and BALB/c) and hamsters infected with different doses of *L. donovani*. Data are expressed as either the number of parasites/1000 host cell nuclei/organ (a and b) or as LDU/organ (c and d). □, BALB/c; ◇, B10; ■, hamster.

TABLE 2

*Maximim parasite burdens on day 14 postinfection in spleen, liver and bone marrow of mice (B10 and BALB/c) and hamsters*

| | Hamster | B10 | BALB/c |
|---|---|---|---|
| N | | | |
| Spleen | $>2\times10^3$ | $5\times10^2$ | $3\times10^2$ |
| Liver | $3\times10^3$ | $3\times10^3$ | $3\times10^3$ |
| Bone marrow | $2\times10^3$ | $1\times10^3$ | $2\times10^2$ |
| LDU | | | |
| Spleen | $>8\times10^2$ | $2\times10^2$ | $1\times10^2$ |
| Liver | $>2\times10^4$ | $4\times10^3$ | $4\times10^3$ |

Parasite burdens are expressed as number of parasites per 1000 host cell nuclei (N) and as LDU. The values given for liver and spleen are the plateau burdens for these organs shown in Fig. 1.

(one sixth of the free drug dose) was more effective than free drug treatment ($P < 0.025$). In the hamster, the free and liposomal therapies were, in spite of the 6 fold difference in the Sb (v) dose, equi-active and gave significant parasite suppression in all 3 sites of infection ($P < 0.001$, Fig. 3). On the basis of the percentage parasite suppressions achieved at the dose levels used here, a species difference in terms of the therapeutic outcome was apparent (Table 3). Either treatment, free or liposomal drug, produced a similar level of suppression in mouse and hamster livers, but in the spleen and bone marrow a significantly greater reduction of parasite burdens was obtained in the hamster. In the hamster no sex difference in the

TABLE 3

*Parasite suppression after acute infection*

| Species | Treatment | *n* | % parasite suppression | | |
|---|---|---|---|---|---|
| | | | Spleen | Liver | Bone marrow |
| Hamster (female) | free drug | 6 | 83 | 91 | 46 |
| | liposomal drug | 6 | 96 | 97 | 91 |
| Hamster (male) | free drug | 7 | 92 | 99 | 96 |
| | liposomal drug | 7 | 96 | 100 | 91 |
| BALB/c mouse | free drug | 5 | 48 | 100 | 49 |
| | liposomal drug | 5 | 52 | 100 | 51 |

The % suppression in parasite numbers in the spleen, liver and bone marrow obtained in *L. donovani*-infected hamsters and BALB/c mice treated with free (total dose 88.8 mg Sb (v)/kg) or liposomal (total dose 14.2 mg Sb (v)/kg) sodium stibogluconate. Animals were drug treated on days 7 and 8 postinfection and burdens assessed on day 14 postinfection.

outcome of therapy was observed and a similar level of parasite suppression was obtained in all 3 sites in male and female animals ($P > 0.05$, Table 3). Similar results have been noted in mice (data not shown).

A delay in drug treatment, i.e. later than day 31 postinfection to allow chronic infections to become established, decreased the effectiveness of free, and to a lesser extent, of liposomal stibogluconate against the visceral disease. In the mouse this decrease in effectiveness was proportional to the chronicity of the infection (Table 4) and in the hamster model (Fig. 4), at day 38 postinfection, neither drug formulation had any suppressive effect on bone marrow burdens compared with controls ($P > 0.05$, Fig. 3). However, there was still a

TABLE 4

*Parasite suppression after chronic infection*

| Day postinfection | Free drug | | | Liposomal drug | | |
|---|---|---|---|---|---|---|
| | Spleen | Liver | BM | Spleen | Liver | BM |
| Day 14 | 64 | 100 | 0 | 40 | 100 | 61 |
| Day 38 | 68 | 89 | 72 | 65 | 93 | 59 |
| Day 55 | | n.d. | | 59 | 100 | 86 |
| Day 56 | 10 | 80 | 40 | 32 | 98 | 34 |
| Day 58 | 8 | 20 | 7 | 51 | 94 | 38 |
| Day 84 | 10 | 42 | 20 | 1 | 79 | 0 |

The parasite suppression (%) achieved in spleen, liver and bone marrow (BM) of *L. donovani*-infected BALB/c mice by treatment with free (total dose 88.8 mg Sb (v)/kg), or liposomal (total dose 14.2 mg Sb (v)/kg) drug. Infected animals were given half the dose indicated at 7 and 6 days before assessment of parasite burdens which was carried out on the day postinfection shown. n.d., not determined.

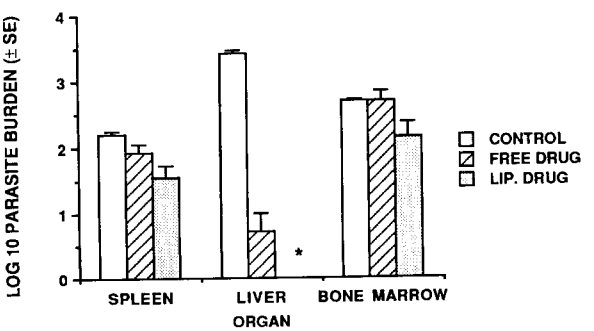


Fig. 2. The effect of free (44.4 mg Sb (v)/kg/day) or liposomal (LIP., 7.1 mg Sb (v)/kg/day) sodium stibogluconate on spleen, liver and bone marrow parasite burdens of *L. donovani*-infected BALB/c mice. Mice were drug-treated on days 7 and 8 postinfection and parasite numbers were assessed 6 days later. *, No parasites found.

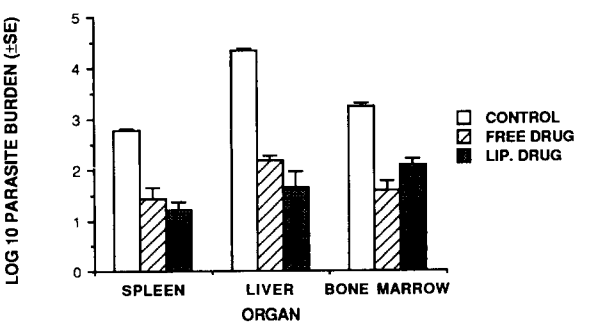


Fig. 3. The spleen, liver and bone marrow parasite burdens of *L. donovani*-infected hamster treated on days 7 and 8 postinfection with either water (controls) or sodium stibogluconate in the free (44.4 mg Sb (v)/kg/day) or liposomal (LIP., 7.1 mg Sb (v)/kg/day) form. Parasite numbers were assessed on day 14 postinfection.

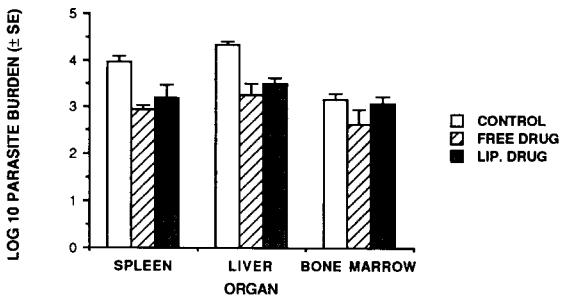


Fig. 4. The spleen, liver and bone marrow parasite burdens of *L. donovani*-infected hamsters treated on days 31 and 32 post-infection with either water (controls) or sodium stibogluconate in the free (44.4 mg Sb (v)/kg/day) or liposomal (LIP., 7.1 mg Sb (v)/kg/day) form. Parasite numbers were assessed on day 38 postinfection.

significant reduction in the number of parasites recovered from the spleen and liver of drug-treated hamsters ($P < 0.001$).

## Discussion

From these results it would appear that the hamster is innately more susceptible to infection with *L. donovani* than the mouse, in the 3 tissues examined at least as expressed as LDU. The number of parasites/1000 host cell nuclei was higher in the spleen and bone marrow of hamster than mice although not in the liver. This may reflect an upper limit of infection for this organ, rather than a difference in susceptibility (Carter et al., 1989c). Nevertheless the total parasite burden of hamsters was greater in all tissues than in mice and yet paradoxically the antiparasitic effects of free and liposomal stibogluconate were greater. Indeed the antiparasitic response in the hamster was greater than in the B10 mouse which expresses a cure H-2 haplotype (Blackwell et al., 1980; Blackwell 1982) and in which the visceral infection responds more readily to liposomal stibogluconate than in the BALB/c, non-cure, mouse (Carter et al., 1989a). However, at the dose levels used, the species difference was only apparent in the spleen and bone marrow and not in the liver, which was the organ most amenable to treatment. These observations provide further evidence for our earlier suggestion (Baillie et al., 1989) that the outcome of chemotherapy in visceral leishmaniasis is the sum of the inherent antileishmanial activity of the drug plus a host factor(s), which can have either a positive or negative value. Thus if we assume that in the hamster the host contribution is positive or much greater than in the mouse, then the greater efficacy of stibogluconate in the former species may be explained. It is perhaps of further significance that the response to drug treatment in the hamster was more variable than in the mouse. In an outbred population, such as the hamster, expression of the host factor(s) may be expected to occur at random whereas the mice are from an inbred population which is homogeneous with respect to visceral leishmaniasis and the response of the infection to chemotherapy.

The present observations in the hamster confirm our earlier results in the mouse that the use of a liposome or niosome formulation increases by (at best) an order of magnitude the apparent efficacy of stibogluconate (Carter et al., 1988; Carter et al., 1989b; Hunter et al., 1988). It would seem that the apparent inaccessibility of spleen and in particular bone marrow parasites to stibogluconate (Carter et al., 1988) is a function of the murine host and that these foci of infection are more readily treated in the hamster. We have, however, been unable to obtain, in either species, the spectacular liposome-mediated increases in the efficacy of Sb (v) against visceral leishmaniasis in the hamster of 900–1000 times (meglumine antimonate, Berman et al., 1986) and some 600 times (meglumine antimonate and stibogluconate, Alving et al., 1978a). There are of course differences in experimental protocol, such as the use of different parasite strains and different routes of drug administration which could explain such variations. Our original hypothesis that the host species difference was the principal source of such discrepancy is negated by the present hamster data. In the hamster and in the mouse the activity of free Sb (v) described here (> 95% suppression of liver parasites at 88.8 mg Sb (v)/kg) is high compared to that described by other workers in hamster models and for comparable suppression of liver parasites, typical literature values are > 1000 mg Sb (v)/kg in the form of meglumine anti-

monate or stibogluconate (Alving et al., 1978a) and 416 mg Sb (v)/kg in the form of meglumine antimonate against a 3-day infection (Berman et al., 1986). In a murine model, Murray et al. (1988) describe a 93% suppression of liver parasites by a dose of free stibogluconate equivalent to 167 mg Sb (v)/kg. The activity of our liposomal stibogluconate is, however, similar to that described by other workers (Alving et al., 1978a; Berman et al., 1986).

The high activity of either formulation of stibogluconate against parasites in the hamster was, however, only apparent against the acute infection (assessed at day 14 postinfection) and the treatments were less effective against a more chronic infection (day 38 postinfection), an effect which has been described in mice (Baillie et al., 1989) and in hamsters (Alving et al., 1984). In untreated control hamsters, parasite burdens at day 38 were, compared to the day 14 values, unchanged in bone marrow but were significantly greater (LDU) in the liver and spleen and it would appear that this increase in total burden would indicate a chronic infectious state with the attendant immunosuppression, which we have suggested in the mouse is antagonistic to the activity of stibogluconate (Baillie et al., 1989). The ready accessibility of bone marrow parasites in the hamster at day 14 postinfection and their apparent inaccessibility at day 38 postinfection lends further support to this suggestion, unless the extreme hepatosplenomegaly in the chronic infection altered drug distribution sufficiently to account for this dramatic change in efficacy. In the hamster, inability to reach these parasites may then not simply be a problem of drug targeting but may, as we have previously suggested (Baillie et al., 1989), be due to a host factor(s) which influences stibogluconate efficacy.

In summary, this study clearly demonstrates the importance of the choice of a model system, which includes an appropriate host animal, for an in vivo assessment of the effects of anti-leishmanial drugs. If for example, the efficacy of stibogluconate treatment had been based purely on liver parasite burdens, the species-dependent drug effect would not have been observed. The difference in efficacy of the treatment regime in hamsters and mice could be the result of a different drug distribution between species, or using our hypothesis (Baillie et al., 1989), to a species-dependent differential expression of a host factor(s), which may contribute negatively or positively to the drug's inherent antiparasitic activity. Experiments are in progress to identify the nature of this host-dependent effect.

## Acknowledgements

This study was supported by the Medical Research Council. J. Alexander holds a Wellcome Trust Lectureship and T.F. Dolan is supported by the University of Strathclyde Research and Development Fund. The donation of pentostam is gratefully acknowledged.

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