J. Pharm. Pharmacol. 1998, 50: 1351–1356
Received June 29, 1998
Accepted August 13, 1998

Comparison of the Efficacy of Free and Non-ionic-surfactant Vesicular Formulations of Paromomycin in a Murine Model of Visceral Leishmaniasis

D. WILLIAMS*†, A. B. MULLEN*, A. J. BAILLIE AND K. C. CARTER*

*Departments of Pharmaceutical Sciences and *Immunology, University of Strathclyde, Glasgow, UK*

Abstract

Non-ionic-surfactant vesicular (NIV) formulations of paromomycin have been tested in-vitro and in-vivo for their activity against *Leishmania donovani*.

Production of NIV was dependent both on the surfactant used and on the concentration of paromomycin; only two of the surfactants studied formed vesicles at the highest paromomycin concentration (9 mg mL^{-1}). At surfactant–lipid concentrations $\geq$1·5 mM, suspensions of NIV (drug- or glucose-loaded) were cytotoxic to macrophages infected with *L. donovani*; high levels of nitrite were produced in cell supernatants. At surfactant–lipid concentrations < 1·5 mM, drug-loaded NIV were more effective than the same dose of free drug, in terms of the percentage of cells infected and the number of parasites/cell. At surfactant–lipid concentrations $\leq$0·15 mM, drug-loaded NIV were ineffective in-vitro. In-vivo, treatment with decaethylene glycol mono *n*-hexadecyl ether paromomycin NIV was more effective than hexaethylene glycol mono *n*-hexadecyl ether paromomycin NIV, in terms of suppression of liver and spleen parasite burdens. Against liver parasites, both types of paromomycin-loaded NIV were more effective than free drug. Neither the NIV nor free forms of paromomycin caused significant suppression of bone-marrow parasites.

The study shows that entrapment of paromomycin in NIV can be used to increase its antileishmanial activity in-vitro and in-vivo.

Delivery systems such as liposomes or non-ionic surfactant vesicles (NIV) can enhance the efficacy of entrapped drug by altering its pharmacokinetic profile, so that a lower dose or a lower number of doses is required to have the same effect as free drug. The clinical advantage of such systems is exemplified by the liposomal formulation of amphotericin B (AmBisome; Tollemar & Ringden 1995) which, because of its lower toxicity, can be used at dose levels normally associated with severe side-effects.

Compared with NIV, liposomes have a number of disadvantages—the constituent phospholipids are readily hydrolysed at the ester linkages between the glycerol backbone and the acyl groups (Grit et al 1993a, b) and peroxidized at unsaturated acyl sites (Konings 1984). Previous studies (Carter et al 1988; Williams et al 1995) have concentrated on an NIV formulation of the antileishmanial drug sodium stibogluconate. The same rationale can be applied to other antileishmanial drugs. The aminoglycoside antibiotic paromomycin (aminosidine) is active against cutaneous and visceral leishmaniasis (Berman 1997). In India combination therapy (paromomycin, 12 mg kg^{-1}, sodium stibogluconate, 20 mg Sb^{v} kg^{-1}) cured 83% of visceral leishmaniasis patients (Thakur et al 1992). However, a recent study has shown that paromomycin at daily doses of 16 or 20 mg kg^{-1} for 21 days was more effective than sodium stibogluconate at 20 mg Sb^{v} kg^{-1} daily for 30 days (Jha et al 1998).

Like other aminoglycosides, paromomycin is used as the sulphate and its high water solubility means that it has to be given parenterally because it is poorly absorbed orally and it is rapidly excreted with a half life of approximately 2 h, which necessitates giving extended treatment courses in the clinic. Thus it has a similar pharmacokinetic profile to sodium stibogluconate.

Because of the high antileishmanial activity of liposomal and NIV forms of sodium stibogluconate

† Present address: Core Technology Ltd, Moorfield Industrial Estate, Kilmarnock, UK.
Correspondence: K. C. Carter, Department of Immunology, The Todd Centre, 31 Taylor Street, Glasgow G4 ONR, UK.

in animal models of visceral leishmaniasis, an NIV formulation of paromomycin should be more effective than the free form. In addition, an NIV formulation of a second highly water-soluble antileishmanial drug, which has a different mode of action from sodium stibogluconate, would widen the applicability of this drug-delivery strategy in visceral leishmaniasis. This study investigates these hypotheses in-vitro and in-vivo using a murine model of visceral leishmaniasis.

Materials and Methods

Materials

Paromomycin sulphate (0·737 mg base mg^{-1}, Sigma data) was obtained from Sigma. Throughout, paromomycin doses are expressed as the base. The surfactants triglycerol monostearate and hexaglycerol distearate were purchased from Blagden Chemicals, UK and the surfactants diethylene glycol mono *n*-hexadecyl ether, tetraethylene glycol mono *n*-hexadecyl ether, hexaethylene glycol mono *n*-hexadecyl ether and decaethylene glycol mono *n*-hexadecyl ether were purchased from Chesham Chemicals, UK. Dicetyl phosphate and ash-free cholesterol were from Sigma. Other reagents were of analytical grade.

Animals and parasites

Experiments were performed on age-matched BALB/c mice (in-house inbred female), 8–10 weeks. In-house bred Golden Syrian hamsters (*Mesocricetus auratus*) were used for maintenance of *Leishmania donovani* (strain MHOM/ET/67 : LV82). Mice were infected by intravenous injection (tail vein, no anaesthetic) of $1-2 \times 10^7$ *L. donovani* amastigotes (Carter et al 1988). The day of parasite administration to the mice was designated day 0 of the experiment.

Preparation of NIV suspensions

Surfactant–lipid mix, typically 750 μmol, consisting of different molar ratios of surfactant, cholesterol and dicetyl phosphate was melted by heating at 130°C for 5 min. The molten mixture was cooled to 70°C, hydrated with preheated glucose solution (300 mM, 5 mL), with pH 7·4 phosphate-buffered saline (PBS—control NIV), or with paromomycin sulphate solution, and homogenized by means of a Model L4R SU Silverson mixer (Silverson Machines, UK), fitted with a 5/8″ tubular workhead. In some experiments a higher concentration of surfactant–lipid was used to prepare NIV suspensions which were then diluted with water just before use to give a final concentration of 150 mM lipid. Vesicle suspensions were stored at 4°C until used. NIV were sized by photon-correlation spectroscopy using a Malvern Zetasizer 4 (Malvern Instruments, UK).

Determination of drug entrapment

Unentrapped drug was removed from paromomycin NIV suspensions by gel filtration on an 18 cm × 2·6 cm Sephadex G50 column with PBS, pH 7·4, as eluent. The gel-filtered NIV suspension (0·1 mL) was added to an equal volume of *n*-propanol, to disrupt the vesicles, and was filter-sterilized. Dilutions of this solution were prepared with sterile water. Paromomycin concentrations were determined by an agar-diffusion inhibition assay of the growth of *Staphylococcus aureus* (NTCC 8532) on sensitivity test agar. Disrupted, diluted paromomycin NIV suspensions or paromomycin standard solutions (0·15 mL) were dispensed into wells cut in the agar. All solutions contained the same concentration of propanol. After overnight incubation of the plates at 31°C the zone of inhibition was measured for each sample. A straight-line relationship was obtained between drug concentration and zone of inhibition (mm). The amount of drug present for a vesicular sample was determined from a standard curve.

In-vitro studies

Complete medium (RPMI 1640 supplemented with 10% v/v foetal calf serum, 100 μg mL^{-1} penicillin, 100 μg mL^{-1} streptomycin and 100 μg mL^{-1} L-glutamine; 5 mL) was used to harvest the cells from the peritoneal cavity of a mouse. Cells from 3 or 4 mice were pooled, washed by centrifugation, and the number of live cells determined by trypan-blue exclusion. Cell suspensions were adjusted to $1-2 \times 10^6$ cells mL^{-1} and 1-mL volumes added to each well of a 24-well tissue-culture plate which contained round 13-mm cover slips. After 2-h incubation at 37°C in an atmosphere of 95% O_2–5% CO_2, the medium was removed from each well and replaced with 0·9 mL fresh medium. The number of cells in the pooled supernatant was counted and the mean number of cells attached/well was determined by subtraction.

L. donovani amastigotes (in 0·1 mL complete medium), obtained from a hamster spleen, were added to each well at different parasite/cell ratios and incubation continued for 1–2 h as above. The medium was then removed from each well and replaced with fresh medium (RPMI 1640 without phenol red, supplemented with 10% v/v foetal calf serum, 100 μg mL^{-1} penicillin, 100 μg mL^{-1} streptomycin and 100 μg mL^{-1} L-glutamine, controls; 1 mL), with medium containing different

concentrations of filter-sterilized free or vesicular paromomycin, or control NIV (glucose-loaded).

Cells were then incubated for a further 48 or 72 h. The cover slips were washed with PBS, fixed in methanol and stained with 10% Giemsa. Microscopic examination of the stained cover slips was used to determine the number (%) of 200 randomly chosen cells infected and, for the first 20 infected cells, the mean number of parasites/infected cell. The supernatant medium from each well was stored in labelled Eppendorf tubes at −20°C for determination of nitrite by use of Greiss reagent (Palmer et al 1987).

Drug treatment and determination of parasite numbers

On days 7 or 7 and 8 postinfection, groups of infected mice (n = 5) were treated, via the tail vein, with 50, 100 or 200 μL PBS (controls), paromomycin solution or NIV suspension. On day 7 post-treatment, parasite burdens in the liver, spleen and bone marrow of control and drug-treated mice were determined (Carter et al 1988). Leishman–Donovan units (LDU) were calculated per organ for the liver and spleen by use of the formula (Bradley & Kirkley 1977):

$$\text{LDU} = \text{amastigote number per 1000 host} - \text{cell nuclei} \times \text{organ weight (g)}$$

Presentation and statistical analysis of data

Parasite suppression (%, mean ± s.e.m.) was determined for a particular site by comparing each experimental value of parasite burden with the relevant mean control value. Parasite burdens were analysed by use of Student's unpaired *t*-test on the $\log_{10}$-transformed data (LDU/organ for spleen and liver and number of parasites/1000 host cell nuclei for bone marrow).

Results

Formation of vesicles comprising surfactant–cholesterol (5 : 4 molar) failed in the presence of paromomycin solution. With some surfactants (hexaglycerol distearate, tetraethylene glycol mono-*n*-hexadecyl ether, hexaethylene glycol mono-*n*-hexadecyl ether and decaethylene glycol mono *n*-hexadecyl ether) admixture of dicetyl phosphate (surfactant–cholesterol–dicetyl phosphate, 5 : 4 : 1 molar) enabled vesicle formation, although paromomycin entrapment was low. Entrapment was improved by increasing the dicetyl phosphate NIV content (5 : 4 : 2 molar) but in the presence of high concentrations of paromomycin NIV of this composition could be formed only by use of the surfactants hexa- and decaethylene glycol mono *n*-hexadecyl ether.

Typical values for drug entrapment and mean vesicle diameter are shown in Table 1. The mean diameters of drug-loaded hexaethylene glycol mono *n*-hexadecyl ether NIV suspensions were much larger than those formed using the decaethylene analogue (Table 1). In general, after gel filtration mean vesicle diameter and variation around the mean was lower, presumably because of the removal of large vesicles from the suspension by the column. This effect of gel filtration was more obvious with hexaethylene glycol mono *n*-hexadecyl ether NIV suspensions than with the decaethylene analogues. Hexaethylene glycol mono *n*-hexadecyl ether drug-loaded vesicles were larger than glucose loaded NIV whereas the mean diameter of glucose- and paromomycin-loaded decaethylene glycol mono *n*-hexadecyl ether NIV were similar (Table 1).

In-vitro activity

NIV (drug- or glucose-loaded) containing ≥1·5 mM surfactant–lipid were toxic to cells and high levels

Table 1. The effect of the concentration of hydrating drug on drug entrapment and vesicle size.

Hydrating drug concn (mg mL^{-1})	Hexaethylene glycol mono *n*-hexadecyl ether			Decaethylene glycol mono *n*-hexadecyl ether		
	Drug content (μg mL^{-1})	Size before (nm)	Size after (nm)	Drug content (μg mL^{-1})	Size before (nm)	Size after (nm)
1·125	ND*	2600 ± 142	519 ± 11	22·45	733 ± 106	271 ± 4
2·25	3·94	1060 ± 68	425 ± 3	22·45	227 ± 3	179 ± 5
3·375	17·83	1229 ± 57	540 ± 14	45·60	288 ± 7	209 ± 5
9·0	3·94	2153 ± 275	403 ± 10	40·97	773 ± 59	215 ± 5
Glucose	–	331 ± 6	–	–	230 ± 2	–

A 5 : 4 : 2 molar ratio of hexa- or decaethylene glycol mono *n*-hexadecyl ether–cholesterol–dicetyl phosphate was hydrated with the drug concentrations shown or with 300 mM glucose solution to form non-ionic-surfactant vesicles. Vesicle mean hydrodynamic diameter was determined both before and after gel filtration of surfactant vesicle suspensions. Data are means ± s.d. *Not detected.

of nitrite were measured in cell supernatant (Table 2).

When surfactant–lipid concentrations were < 1·5 mM NIV suspensions were not toxic to infected cells. The antiparasitic activity (number (%) of cells infected and number of parasites/cell, Table 3) of paromomycin NIV was greater than that of the free drug. When surfactant–lipid concentrations were < 0·15 mM NIV suspensions were inactive against parasites and nitrite was not detected.

In-vivo activity

An initial experiment was performed to determine the dose response for free paromomycin against *L. donovani* infection in mice. The maximum dose without overt signs of toxicity to the treated mice was 80 mg kg^{-1}, a dose which failed to clear the liver of parasites (Figure 1). On the basis of paromomycin-mediated parasite suppression, the three tissue sites examined could be ranked liver > spleen > bone marrow.

Paromomycin NIV were more effective than free drug (Table 4). Treatment with decaethylene glycol mono *n*-hexadecyl ether paromomycin NIV (44 or 58 mg kg^{-1}) resulted in almost complete parasite clearance from the liver (99% suppression compared with control value) but less suppression of spleen parasites (66% at 58 mg kg^{-1}). Treatment with hexaethylene glycol mono *n*-hexadecyl ether paromomycin NIV was less effective than with the decaethylene analogue and only 58 mg kg^{-1} hexaethylene glycol mono *n*-hexadecyl ether paromomycin NIV resulted in greater suppression in

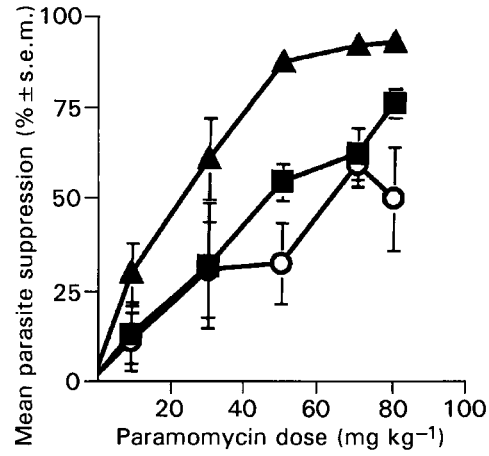

Figure 1. Paromomycin dose–response curves for liver (▲), spleen (■) and bone-marrow (○) parasite burdens of BALB/c mice infected with *L. donovani*. Mice were treated on days 7 and 8 post-infection with phosphate-buffered saline (controls) or the total paromomycin dose shown and parasite burdens were determined on day 14. Control parasite burdens: spleen 39 ± 8, liver 3068 ± 168, bone marrow 295 ± 59.

Table 2. The effect of different drug formulations on nitrite levels in supernatant from *L. donovani*-infected macrophages.

Treatment	Supernatant nitrite concentration (μM) at treatment dilution levels:		
	1 : 10	1 : 100	1 : 1000
Hexaethylene glycol mono *n*-hexadecyl ether–glucose	19·7 ± 0·91	ND*	ND
Hexaethylene glycol mono *n*-hexadecyl ether–paromomycin	24·2 ± 6·4	10·8 ± 7·1	1·3 ± 0·1
Decaethylene glycol mono *n*-hexadecyl ether–glucose	9·9 ± 0·7	ND	ND
Decaethylene glycol mono *n*-hexadecyl ether–paromomycin	12·0 ± 0·2	2·3 ± 0	1·3 ± 0·1
Free paromomycin	0·9 ± 0	1·3 ± 0·1	1·3 ± 0·1

Macrophages were infected by incubation at a cell/amastigote ratio of 2 : 1 for 1 h. Infected cells were exposed for 72 h to glucose-loaded non-ionic-surfactant vesicles or to paromomycin solution (free) or to a paromomycin–non-ionic-surfactant vesicle suspension (vesicle composition: surfactant–cholesterol–dicetyl phosphate, 5 : 4 : 1 molar). Non-ionic-surfactant vesicle suspensions comprising 150 mM surfactant–lipid and 1000 μg mL^{-1} paromomycin or 300 mM glucose were incubated with the cells at final dilutions of 1 : 10, 1 : 100, 1 : 1000. Cells were incubated with paromomycin solution at concentrations of 100 μg mL^{-1} (1 : 10), 10 μg mL^{-1} (1 : 100) or 1 μg mL^{-1} (1 : 1000). Data are means ± s.e.m. *Not detected.

Table 3. The effect of different drug formulations on the survival of *L. donovani* in macrophages.

Treatment	Macrophage infected (%)	Parasites/macrophage
Control	36 ± 2	4 ± 0·6
Free drug	25 ± 9 (31)	3·2 ± 0·2 (20)
Hexaethylene glycol mono *n*-hexadecyl ether vesicle	6 ± 6 (83)	3·7 ± 0·5 (7·5)
Decaethylene glycol mono *n*-hexadecyl ether vesicle	2 ± 1 (94)	2 ± 1 (50)

Macrophages were infected by incubation at a cell/amastigote ratio of 2 : 1 for 1 h. Infected cells were exposed to medium alone (controls), or to paromomycin (34 μg mL^{-1}) in the form of a solution or a paromomycin-loaded non-ionic-surfactant vesicle suspension (vesicle composition: surfactant–cholesterol–dicetyl phosphate, 5 : 4 : 1 molar, concentration 150 mM) for 48h. Data are means ± s.e.m. Values in parenthesis show the inhibition (%) of that parameter compared with the control.

the liver than did the free drug ($P < 0{\cdot}005$). None of the NIV forms of paromomycin suppressed bone-marrow parasites.

A concentrated decaethylene glycol mono *n*-hexadecyl ether paromomycin NIV suspension diluted just before administration (17·5 mg kg^{-1}), resulted in significant liver parasite suppression, 66 ± 12%, and was more effective than a higher dose (44·5 mg kg^{-1}) of free drug (Table 5).

Discussion

The results of this study showed that an NIV delivery system increased the efficacy of paromomycin against *L. donovani* both in-vitro and in-vivo. In-vitro, NIV suspensions containing ≥1·5 mM surfactant–lipid were toxic to cells; this is, perhaps, not surprising because of the membranolytic activity of non-ionic surfactants. The high levels of nitrite present in the supernatant of macrophages exposed to high concentrations of NIV indicate that these cells produced high levels of nitric oxide. This is not unexpected because nitric oxide production is associated with apoptosis (Ankarcrona et al 1994; Umansky et al 1997). When surfactant–lipid concentrations were < 1·5 mM, NIV suspensions were non-toxic but also ineffective against parasites although, because of the dilution involved, the drug concentration (1 μg mL^{-1}) was sub-parasiticidal.

Of the surfactants tested, the hexaethylene and decaethylene glycol mono *n*-hexadecyl ethers produced the most successful NIV for paromomycin entrapment; decaethylene glycol mono *n*-hexadecyl ether, in particular, gave stable, small mean-diameter NIV suspensions. Diethylene and tetraethylene glycol mono *n*-hexadecyl ethers did not form NIV when the paromomycin concentration was high (9 mg mL^{-1}). Because all of these polyoxyethylene ether surfactants have the same hydrophobic C_{16} alkyl group and differ only in the number of oxyethylene residues (n) in the hydrophilic head group, a positive correlation between head-group size and the ability to form NIV in the presence of paromomycin was apparent. This suggests interaction between paromomycin and the head group which influences vesicle stability. Similar evidence for interaction between sodium stibogluconate and NIV containing the same surfactants was observed by Williams et al (1997),

Table 4. Parasite suppression achieved by treatment with different forms of paromomycin.

Treatment	Parasite suppression (%)		
	Spleen	Liver	Bone marrow
Free drug, 44 mg kg^{-1}	12 ± 7	84 ± 4†	6 ± 5
Free drug, 58 mg kg^{-1}	11 ± 5	73 ± 6†	26 ± 15
Hexaethylene glycol mono *n*-hexadecyl ether vesicle, 44 mg kg^{-1}	26 ± 16	90 ± 2†	24 ± 14
Hexaethylene glycol mono *n*-hexadecyl ether vesicle, 58 mg kg^{-1}	46 ± 18	98 ± 1†‡	26 ± 8
Decaethylene glycol mono *n*-hexadecyl ether vesicle, 44 mg kg^{-1}	50 ± 17	99 ± 0†‡	20 ± 9
Decaethylene glycol mono *n*-hexadecyl ether vesicle, 58 mg kg^{-1}	66 ± 4*	99 ± 0†‡	20 ± 9

BALB/c mice infected with *L. donovani* were treated on days 7 and 8 with phosphate-buffered saline (controls) or paromomycin (44 or 58 mg kg^{-1}) either as a solution or as a non-ionic-surfactant vesicle suspension (surfactant–cholesterol–dicetyl phosphate, 5 : 4 : 2 molar, vesicle concentration 150 mM). Parasite burdens were determined on day 14. Data are means ± s.e.m. *$P < 0{\cdot}025$; †$P < 0{\cdot}0005$, significantly different from control result; ‡$P < 0{\cdot}0005$, significantly different from result for free drug.

Table 5. Parasite suppression achieved by treatment with different forms of paromomycin.

Treatment	Parasite suppression (%)		
	Spleen	Liver	Bone marrow
Free drug, 70·2 mg kg^{-1}	29 ± 14	51 ± 6†	22 ± 10
Free drug, 44·5 mg kg^{-1}	20 ± 5	24 ± 14*‡	20 ± 7
Hexaethylene glycol mono *n*-hexadecyl ether vesicle, 17·5 mg kg^{-1}	20 ± 20	66 ± 12†	16 ± 8

BALB/c mice infected with *L. donovani* were treated on day 7 with a single dose of paromomycin solution or with non-ionic-surfactant vesicle suspension (decaethylene glycol mono *n*-hexadecyl ether–cholesterol–dicetyl phosphate, 5 : 4 : 2 molar, vesicle concentration 150 mM). Surfactant lipid mix (3750 μmol) was hydrated with paromomycin solution (39·5 mg mL^{-1}, 5 mL) then diluted to a paromomycin concentration of 7·9 mg mL^{-1}. Parasite burdens were determined on day 14. Data are means ± s.e.m. *$P < 0{\cdot}01$, †$P < 0{\cdot}005$, significantly different from control result; ‡$P < 0{\cdot}025$, significantly different from result for non-ionic-surfactant vesicle suspension.

who reported that mean vesicle diameter was solute-dependent and sensitive to drug concentration. Further evidence of the role of the head group in the formation of paromomycin NIV was seen with the polyglycerol stearates—hexaglycerol distearate could be used to form paromomycin NIV suspensions whereas triglycerol monostearate could not.

In-vivo, free paromomycin at a total dose of 70 mg kg^{-1} (equivalent to 113·7 mmol base kg^{-1}) was active (90–95% suppression) but failed to clear the liver of *Leishmania* parasites. Against spleen and bone-marrow parasites it was less effective. Higher doses were not tested because of toxicity, and presumably to cure BALB/c mice a multiple dosing regimen would be required. In the same animal model, at a comparable dose level, free sodium stibogluconate (113·7 μmol Sb^v kg^{-1} or 312 μg Sb^v/mouse) resulted in 50% suppression of liver parasite burdens (Baillie et al 1987). These findings support those of Jha et al (1998) that paromomycin is more active than sodium stibogluconate. However sodium stibogluconate has a greater therapeutic index than paromomycin and mice can tolerate much higher doses of sodium stibogluconate without overt toxicity. Complete suppression of liver parasites is achieved at a total dose of 2 mg Sb^v/mouse (Baillie et al 1987).

The increased efficacy of vesicular paromomycin was most obvious with decaethylene glycol mono *n*-hexadecyl ether NIV—a dose of 44 mg kg^{-1} cleared 99% of liver parasites. The activity of paromomycin NIV against spleen and bone-marrow parasites was disappointing—compared with controls the only significant suppression was for decaethylene glycol mono *n*-hexadecyl ether NIV in the spleen at the high paromomycin dose (58 mg kg^{-1}). Bone-marrow parasites were refractory at the dose levels used. Parasite susceptibility to paromomycin, free or vesicular, followed the same site-specific trend—liver parasites > spleen parasites > bone marrow parasites—already described for sodium stibogluconate (Carter et al 1988) and amphotericin B (Mullen et al 1997). This provides further evidence that the site-specific response to treatment of murine visceral leishmaniasis is not drug-dependent but rather reflects the difficulty of accessing some anatomical sites of infection.

The results of this study demonstrate that non-ionic-surfactant vesicular forms of paromomycin are more active than free drug in an experimental model of visceral leishmaniasis. Although it further widens the range of drugs to which the NIV system is applicable, it is apparent that such NIV formulations need to be drug-specific.

Acknowledgements

This investigation received financial support from the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (TDR).

References

Ankarcrona, M., Dypbukt, J. M., Brune, B., Nicotera, P. (1994) Interleukin-1β-induced nitric oxide production activates apoptosis in pancreatic RINm5F cell. Exp. Cell Res. 213: 172–177

Baillie, A. J., Coombs, G. H., Dolan, T. F., Hunter, C. A., Laakso, T., Sjöholms, I., Stjärnkvist, P. (1987) Biodegradable microspheres: polyacryl starch microparticles as a delivery system for the antileishmanial drug, sodium stibogluconate. J. Pharm. Pharmacol. 39: 832–835

Berman, J. D. (1997) Human leishmaniasis: clinical, diagnostic, and chemotherapeutic developments in the last 10 years. Clin. Infect. Dis. 24: 684–703

Bradley, D. J., Kirkley, J. (1977) Regulation of *Leishmania* populations within the host. I The variable course of *Leishmania donovani* infections in mice. Clin. Exp. Immunol. 30: 119–129

Carter, K. C., Baillie, A. J., Alexander, J., Dolan, T. F. (1988) The therapeutic effect of sodium stibogluconate in BALB/c mice infected with *Leishmania donovani* is organ-dependent. J. Pharm. Pharmacol. 40: 370–373

Grit, M., Underberg, W. J. M., Crommelin, D. J. A. (1993a) Hydrolysis of saturated soybean phosphatidylcholine in aqueous liposome dispersions. J. Pharm. Sci. 82: 262–366

Grit, M., Zuidam, N. J., Underberg, W. J. M., Crommelin, D. J. A. (1993b) Hydrolysis of partially saturated egg phosphatidylcholine in aqueous liposome dispersions and the effect of cholesterol incorporation on hydrolysis kinetics. J. Pharm. Pharmacol. 45: 490–495

Jha, T. K., Olliaro, P., Thakur, C. P., Kanyok, T. P., Singhania, B. L., Singh, I. J., Singh, N. K., Akhoury, S., Jha, S. (1998) Randomized controlled trial of aminosidine (paromomycin) v sodium stibogluconate for treating visceral leishmaniasis in North Bihar, India. Br. Med. J. 316: 1200–1205

Konings, A. W. T. (1984). Lipid peroxidation in liposomes. In: Gregoriadis, G. (ed.) Liposome Technology. 1st edn, Boca Raton, CRC Press, pp 139–161

Mullen, A. B., Carter, K. C., Baillie, A. J. (1997) A comparison of the efficacy of various formulations of amphotericin B against murine visceral leishmaniasis. Antimicrob. Agents Chemother. 41: 2089–2092

Palmer, R. M. J., Ferrige, A. G., Moncada, S. (1987) Nitric oxide release accounts for the biological activity of endothelium-derived relaxant factor. Nature 327: 524–526

Thakur, C. P., Olliaro, P., Gothoskar, S., Bhowmick, S., Choudhury, B. K., Prasad, S., Kumar, M., Verma, B. B. (1992) Treatment of visceral leishmaniasis (kala-azar) with aminosidine (paromomycin)–antimonial combinations, a pilot study in Bihar, India. Trans. R. Soc. Trop. Med. Hyg. 86: 615–616

Tollemar, J., Ringden, O. (1995) Formulations of Amphotericin B. Drug Safety 13: 207–218

Umansky, V., Bucur, M., Schirrmacher, V., Rocha, M. (1997) Activated endothelial cells induce apoptosis in lymphoma cells: role of nitric oxide. Int. J. Oncol. 10: 465–471

Williams, D. M., Carter, K. C., Baillie, A. J. (1995) Visceral leishmaniasis in the BALB/c mouse: a comparison of the in vivo activity of five non-ionic surfactant vesicle preparations of sodium stibogluconate. J. Drug Targeting 3: 1–7