

# In Vitro and In Vivo Interactions between Miltefosine and Other Antileishmanial Drugs

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**The interaction of miltefosine with amphotericin B, sodium stibogluconate, paromomycin, and sitamaquine was assessed in vitro and additionally for the first three combinations in vivo. In vitro interactions were indifferent for miltefosine combined with amphotericin B (mean sums of fractional inhibitory concentrations [mean  $\Sigma$ FICs] ranging from 1.22 to 1.51 at the 50% effective concentration [EC<sub>50</sub>] level and 1.08 to 1.38 at the EC<sub>90</sub> level), sitamaquine (mean  $\Sigma$ FICs from 1.33 to 1.38 and 1.0 to 1.02, respectively), and paromomycin (mean  $\Sigma$ FICs from 0.79 to 0.93 at the EC<sub>50</sub> and 0.77 to 1.35 at the EC<sub>90</sub> level). Some synergy was observed for miltefosine combined with sodium stibogluconate (mean  $\Sigma$ FICs from 0.61 to 0.75 at EC<sub>50</sub> and 0.49 to 0.97 at EC<sub>90</sub>). Different interactions were found in vivo, where the highest potentiation of miltefosine activity was achieved with amphotericin B (activity enhancement index [AEI] of up to 11.3). No significant interaction was observed when miltefosine was combined with sodium stibogluconate (AEI of up to 2.38). The potentiation of miltefosine in vivo was also achieved with the combination of miltefosine and paromomycin (AEI of up to 7.22).**

Visceral leishmaniasis (VL) is one of several manifestations of disease caused by protozoan parasites belonging to the genus *Leishmania*. The causative species of VL are *Leishmania donovani* on the Indian subcontinent and East Africa, *Leishmania infantum* in the Mediterranean region, and *Leishmania chagasi* in the New World, with the latter two species having a close genetic relationship (15). VL is endemic in 62 countries, with a total of 200 million people at risk (15), and coinfection with human immunodeficiency virus poses a major problem in chemotherapy (1). The yearly incidence is estimated at 0.5 million cases (11). Recommended first-line therapies include pentavalent antimonials (Sb<sup>v</sup>) (sodium stibogluconate [Pentostam] and meglumine antimoniate), the polyene antibiotic amphotericin B as well as its liposomal formulation, AmBisome, and the diamidine pentamidine. Drugs currently in clinical trials are the aminoglycoside paromomycin and the 8-aminoquinoline sitamaquine (10). All recommended treatments have limitations. Resistance to Sb<sup>v</sup> has developed in some areas of endemicity, such as the high-prevalence epidemic region of the state of Bihar, where failure rates of up to 65% have been reported and where the use of antimony has been abandoned (45).

Miltefosine (Impavido), an alkylphosphocholine, is the most recent drug to come to the market for the treatment of VL. Cure rates of 95% and 94% were achieved after oral administration in phase II and phase III trials in Indian patients, leading to its registration in India in 2002 (17, 44). In a compassionate-use program involving 39 patients with human immunodeficiency virus-leishmaniasis coinfection, initial parasitological cure was observed in 41% of patients (41). Although miltefosine has the advantage of being an effective oral drug, its use in women of child-bearing age is restricted due to teratogenicity, which has been observed in one species (rat) with a no-effect dose level of 0.6 mg/kg. In addition, miltefosine

has a long half-life, which might encourage the emergence of resistance once its use becomes widespread (5). In laboratory studies, miltefosine-resistant clones of *L. donovani* were selected (40), and resistance was shown to be related to two mutations on an aminophospholipid translocase (32).

Drug combinations that aim to delay or prevent the emergence of resistance, increase efficacy, or shorten the course of treatment are the standard in the treatment of several viral, bacterial, and parasitic infections (31, 49). In VL, the combination of paromomycin plus sodium stibogluconate (8, 28, 39, 47) as well as sodium stibogluconate combined with allopurinol (7, 42) have been investigated experimentally and clinically. Combination therapy for leishmaniasis could have a role in delaying the development of resistance and shortening the duration of treatment (43). Here, we report on the in vitro and in vivo interactions between miltefosine and other antileishmanial drugs. The aim of this study was to acquire baseline data for a rational approach to identify miltefosine combinations for the therapy of VL.

## MATERIALS AND METHODS

**Parasites.** *Leishmania donovani* strain MHOM/ET/67/L82 was used throughout the study. The strain was maintained in Syrian hamsters (*Mesocricetus auratus*), and amastigotes were harvested from the spleen of an infected animal.

**Drugs.** Miltefosine was obtained from Zentaris GmbH (Frankfurt am Main, Germany) (formerly Asta Medica). Sodium stibogluconate was a gift from GlaxoSmithKline (Dartford, United Kingdom), and WR6026 (sitamaquine) was obtained from WRAIR (Washington, D.C.). Amphotericin B deoxycholate (Fungizone) was obtained from E.R. Squibb and Sons (Hounslow, United Kingdom), and paromomycin sulfate was purchased from Sigma (Poole, United Kingdom).

For in vitro assays, 10 mM stock solutions were prepared for miltefosine in deionized water and sitamaquine dihydrochloride in dimethyl sulfoxide (DMSO), and a 5.4 mM stock solution was prepared for amphotericin B deoxycholate in water. Sodium stibogluconate was suspended at 10 mg Sb<sup>v</sup>/ml in DMSO, and paromomycin sulfate was suspended at 100 mM. All subsequent dilutions were prepared in the respective culture medium fresh on the day of the assay. Miltefosine and amphotericin B deoxycholate stock solutions were sterilized by passage through a filter (0.2- $\mu$ m membrane). Suspensions in DMSO were rotated on a Spiramax (Denley, Sussex, United Kingdom) apparatus with glass beads over-

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night prior to the assay to ensure solubility. The final DMSO concentration never exceeded 0.2% and had no deleterious effect on parasite growth.

For the *in vivo* part of the study, sodium stibogluconate was dissolved in phosphate-buffered saline, and miltefosine and paromomycin sulfate were dissolved in deionized water. Amphotericin B deoxycholate was reconstituted according to the manufacturer's directions by rapidly expressing 10 ml water into the lyophilized cake and shaking the vial until the colloidal suspension became clear. Further dilutions were prepared in 5% glucose.

**Determination of drug interactions against intracellular amastigotes in peritoneal macrophages *in vitro*.** *In vitro* drug interactions were assessed using a modified fixed-ratio isobologram method (14). Briefly, predetermined 50% effective concentration ( $EC_{50}$ ) values were used to decide the top concentrations of the individual drugs to ensure that the  $EC_{50}$  fell near the midpoint of a six-point twofold dilution series. Top concentrations used were 40  $\mu$ M for miltefosine and 0.2  $\mu$ M for amphotericin B deoxycholate in a 72-h assay, 20  $\mu$ M for miltefosine in a 120-h assay (interaction assays with miltefosine-sodium stibogluconate and miltefosine-sitamaquine dihydrochloride) and a 168-h assay (interaction assay with miltefosine-paromomycin sulfate), 20  $\mu$ M for sitamaquine (120-h assay), 200  $\mu$ M for paromomycin sulfate (168-h assay), and 20  $\mu$ g/ml Sb<sup>v</sup> for sodium stibogluconate (120-h assay). The top concentrations were used to prepare fixed-ratio solutions at ratios of 5:0, 4:1, 3:2, 2:3, 1:4, and 0:5 of miltefosine and the partner drug, which were serially diluted six times in twofold dilutions.

Peritoneal macrophages from CD1 mice (Charles River Ltd., Margate, United Kingdom) were harvested by lavage with ice-cold RPMI 1640 medium (Sigma, Poole, United Kingdom) 24 h after induction with 2% soluble starch (Sigma, Poole, United Kingdom). Macrophages were diluted to  $5 \times 10^5$  cells/ml in RPMI 1640 medium plus 10% heat-inactivated fetal calf serum, plated in 16-well Lab-Tek tissue culture chamber slides (Scientific Laboratory Supplies, Wilford, United Kingdom) at a plating density of  $5 \times 10^4$  macrophages/well, and allowed to adhere overnight at 37°C in a 5% CO<sub>2</sub>-95% air mixture. Macrophages were infected with amastigotes at a macrophage-amastigote ratio of 1:5. Infected cultures were maintained at 37°C in a 5% CO<sub>2</sub>-95% air mixture. After 20 h, extracellular parasites were removed by washing, and fresh medium containing the different fixed-ratio solutions and their dilutions or no drug was added. Each point was tested in triplicate. Interaction assay mixtures were incubated at 37°C in a 5% CO<sub>2</sub>-95% air mixture for the times indicated above. Further medium changes with fresh drug were carried out after 72 h in the 120-h assays and after 72 h and 120 h in the 168-h assays. Drug activity was determined from the percentage of infected cells in drug-treated cultures in relation to nontreated cultures after methanol fixation and Giemsa staining. From the known concentrations of miltefosine and the partner drug in the fixed-ratio solutions,  $EC_{50}$  and  $EC_{90}$  values were calculated by sigmoidal analysis using MicroSoftXlfit (ID Business Solution, Guildford, United Kingdom). For each of the drugs, an  $EC_{50}$  and an  $EC_{90}$  on its own were obtained from the fixed-ratio solutions at ratios of 5:0 and 0:5. Solutions at ratios of 4:1, 3:2, 2:3, and 1:4 yielded the  $EC_{50}$  and  $EC_{90}$  of each of the drugs in combination (14).

Three separate experiments were performed *in vitro*.

**Determination of FIC index and isobologram construction.** Fractional inhibitory concentrations (FICs) and sum FICs ( $\Sigma$ FICs [FIC miltefosine + FIC partner drug]) were calculated as follows (2): FIC of miltefosine =  $EC_{50(90)}$  of miltefosine in combination/ $EC_{50(90)}$  of miltefosine alone. The same was applied to the partner drug. FICs and  $\Sigma$ FICs were calculated for all fixed-ratio solutions, and FICs were used to construct isobolograms. Mean  $\Sigma$ FICs were used to classify the nature of the interaction.

**Assessment of drug interactions *in vivo* using a checkerboard design.** Female BALB/c mice (ordered at 20 g; Charles River Ltd., Margate, United Kingdom) were infected intravenously with  $2 \times 10^7$  *L. donovani* MHOM/ET/67/L82 amastigotes and randomly sorted into groups of five. Mice were dosed 7 days postinfection for five consecutive days and sacrificed 3 days after the completion of treatment (day 14 postinfection). Groups of mice were weighed before and after treatment, and the percent weight change was recorded. Impression smears were prepared from weighed livers, methanol fixed, and stained with 10% Giemsa stain in water. The number of amastigotes per 500 liver cell nuclei was determined and multiplied by the liver weight in milligrams to obtain Leishman-Donovan units (3). The percent inhibition was calculated for all drug-treated groups in relation to a nontreated group, and  $ED_{50}$ s were calculated by sigmoidal regression analysis using MicroSoftXlfit (ID Business Solution, Guildford, United Kingdom).

The dosing scheme followed a checkerboard design. Both miltefosine and the partner drugs were diluted threefold to obtain three different dose levels. All possible combinations of the doses used were tested with a group of five mice for every combined dose and control (16 groups of mice per combination in total).

Miltefosine was administered orally at 15, 5, and 1.6 mg per kg of body weight per dose in combination with sodium stibogluconate and at 9, 3, and 1 mg/kg/dose in subsequent combination experiments. Sodium stibogluconate was administered subcutaneously at 30, 10, and 3.3 mg of Sb<sup>v</sup>/kg/dose; amphotericin B deoxycholate was administered intravenously by slow infusion at 0.5, 0.16, and 0.05 mg/kg/dose; and paromomycin sulfate was administered subcutaneously at 63, 21, and 7 mg/kg/dose.

The miltefosine-sodium stibogluconate combination was tested twice. The top doses used in the first experiment were highly active (30 mg/kg for miltefosine and 45 mg/kg for Sb<sup>v</sup>), and doses were lowered in the subsequent experiment as described above. The combinations of miltefosine with amphotericin B and paromomycin sulfate were tested once.

**Analysis of drug combinations *in vivo*.** Where applicable, isobolograms were constructed by plotting the  $ED_{50}$  of miltefosine against the respective dose of the partner drug and vice versa (33). Additionally, an activity enhancement index (AEI) was determined (34), which was calculated as follows:  $ED_{50}$  of miltefosine alone/ $ED_{50}$  of miltefosine in combination. The AEI was calculated for each dose level of the partner drug. An AEI of >1 indicates activity enhancement, and an AEI of <1 indicates activity reduction. An AEI of less than 2.0 was considered insignificant (34).

## RESULTS

**Sensitivities of intracellular amastigotes *in vitro*.** A summary of individual  $EC_{50}$  and  $EC_{90}$  values with the times of incubation required to give suitable dose response effects at both levels is given in Table 1.  $EC_{50}$ s of miltefosine ranged from 10.78  $\mu$ M at 3 days to 2.21  $\mu$ M and 2.68  $\mu$ M at 5 and 7 days, respectively, in repeated experiments. A representative value at 5 days is shown in Table 1. The infection level (percentage of infected macrophages) in untreated control cultures is indicated. Toxicity towards macrophages was observed with miltefosine above 40  $\mu$ M in a 3-day assay and 20  $\mu$ M in a 5-day assay and with sitamaquine above 20  $\mu$ M in a 5-day assay. No concentrations toxic to macrophages were used in this study.

**Drug interactions *in vitro* against intracellular amastigotes in peritoneal macrophages.** *In vitro* interactions were assessed using a modified fixed-ratio isobologram method, and data were analyzed at the  $EC_{50}$  and  $EC_{90}$  levels. Mean  $\Sigma$ FICs are presented in Table 2 for two independent experiments. Representative isobolograms are shown in Fig. 1. Interactions were classified as synergistic with mean  $\Sigma$ FICs of  $\leq 0.5$ , as antagonistic with mean  $\Sigma$ FICs of  $> 4$ , and as indifferent with mean  $\Sigma$ FICs between  $> 0.5$  and  $\leq 4$ . The interaction of miltefosine with amphotericin B deoxycholate and sitamaquine dihydrochloride was indifferent with mean  $\Sigma$ FICs of 1.22 to 1.51 and 1.33 to 1.38 at the  $EC_{50}$  level and 1.08 to 1.38 and 1.02 at the  $EC_{90}$  level, respectively. The interaction of miltefosine with sodium stibogluconate was classified as indifferent to synergistic with mean  $\Sigma$ FICs of 0.61 to 0.75 ( $EC_{50}$ ) and 0.49 to 0.97 ( $EC_{90}$ ). Miltefosine interaction with paromomycin sulfate displayed indifference (mean  $\Sigma$ FICs of 0.79 to 0.93 and 0.77 to 1.35, respectively, at both activity levels).

**Miltefosine in combination with selected partner drugs *in vivo*.** Interactions between miltefosine and sodium stibogluconate, amphotericin B deoxycholate, and paromomycin sulfate were determined *in vivo*. AEIs are summarized in Table 3. Miltefosine combined with the top dose of 0.5 mg/kg/dose amphotericin B deoxycholate gave the highest index of 11.31, followed by paromomycin sulfate at the top dose of 63 mg/kg/dose with an index of 7.22. The index for miltefosine combined with 30 mg Sb<sup>v</sup>/kg/dose was insignificant, with an index of 2.38. A graphical representation (isobologram) for the combinations

TABLE 1. Activity of individual drugs against intracellular amastigotes<sup>d</sup>

Drug	Infection level <sup>a</sup> (%)	Days <sup>b</sup>	EC <sub>50</sub> (μM)	EC <sub>90</sub> (μM)
Amphotericin B	84	3	0.033 (0.033–0.034)	0.080 (0.061–0.099)
Miltefosine	89	5	6.18 (6.01–6.36)	14.47 (11.74–17.2)
WR6026 (sitamaquine)	77	5	5.09 (4.78–5.41)	20.33 (18.48–22.18)
Sodium stibogluconate (Sb <sup>v</sup> )	89	5	2.39 <sup>c</sup> (2.06–2.72)	13.43 (10.47–16.39)
Paromomycin	71	7	40.60 (32.39–48.81)	136.8 (100.8–172.7)

<sup>a</sup> Infection level indicates the percentage of macrophages infected in untreated control cultures at the end point.

<sup>b</sup> Days indicate the number of days of incubation.

<sup>c</sup> Values for sodium stibogluconate are given in micrograms of Sb<sup>v</sup> per milliliter.

<sup>d</sup> All drugs were used in their salt form as stated in the text.

miltefosine-amphotericin B deoxycholate and miltefosine-paromomycin sulfate is given in Fig. 2. The interaction of miltefosine with amphotericin B deoxycholate displayed a concave curve consistently at all dose levels, demonstrating a gradual increase of miltefosine activity with escalating doses of amphotericin B. This is also reflected in the AEI (Table 3). The combination of miltefosine and paromomycin sulfate showed different interactions at different dose levels with a loss of activity when miltefosine was combined with 21 mg/kg of paromomycin, which is reflected in both the isobologram and AEI (Table 3). It has to be noted that the *in vivo* activity of paromomycin sulfate was low, with the ED<sub>50</sub> lying outside the dose range tested.

## DISCUSSION

This is the first systematic study of interactions between antileishmanial drugs. This study aimed to identify drug combinations that can potentiate the activity of miltefosine and described a matrix of patterns of interaction between antileishmanials. The nature of interaction was first determined *in vitro*, and selected combinations were further investigated *in vivo*. Analysis of drug interactions aimed to indicate whether the nature of the interaction could be categorized as synergistic, indifferent, or antagonistic. Definitions and discussions are available for *in vitro* studies for some microbes, but data obtained from animal models are less defined and more difficult to interpret (13, 18). *In vitro* data are based on an extended ratio and concentration range. *In vivo*, the number of manageable doses is limited. For detection of potentiation, choosing the right doses in relation to the level of analysis is crucial. Methods of analyzing the *in vivo* combinations were based on those used for antimalarial drug combinations (33, 34).

Different trends were demonstrated between *in vitro* and *in vivo* data. To classify the interactions, mean ΣFICs of 0.5 and 4 were used as cutoffs. These categories take experimental error ranges based on twofold dilutions into account, which raises questions about the biological relevance of values between 0.5 and 4 (18, 30). All *in vitro* interactions assessed displayed indifference. The same trend of *in vitro* interactions was observed in a study on promastigotes (unpublished data). The interaction of miltefosine and sodium stibogluconate was on the borderline of synergism *in vitro* but showed no poten-

TABLE 2. Mean ΣFICs of interactions between miltefosine and partner drugs towards intracellular amastigotes *in vitro*

Partner drug	Expt	Days <sup>a</sup>	Mean ΣFIC ± SD <sup>b</sup> at EC <sub>50</sub>	Mean ΣFIC ± SD <sup>b</sup> at EC <sub>90</sub>
Amphotericin B	1	3	1.22 ± 0.15	1.38 ± 0.13
	2	3	1.51 ± 0.44	1.08 ± 0.27
Sodium stibogluconate	1	5	0.61 ± 0.10	0.49 ± 0.09
	2	5	0.75 ± 0.05	0.97 ± 0.21
WR6026 (sitamaquine)	1	5	1.38 ± 0.29	1.02 ± 0.29
	2	5	1.33 ± 0.13	1.00 ± 0.25
Paromomycin	1	7	0.79 ± 0.13	0.77 ± 0.22
	2	7	0.93 ± 0.14	1.35 ± 0.31

<sup>a</sup> Days of incubation.

<sup>b</sup> SD, standard deviation.

tiation *in vivo*. Conversely, the interaction of miltefosine and amphotericin B was the most positive *in vivo*. Differences between *in vitro* and *in vivo* data can be expected, as *in vitro* systems measure direct antiparasitic activity, but *in vivo* parasites are exposed to variable levels of drug and metabolites in different tissues.

The activity of miltefosine was also enhanced when it was combined with the top dose of paromomycin *in vivo*. There was a slight decrease in activity over the mid-range of doses. Different interactions at different dose levels have been reported for antimalarial combinations (16, 48). In the case of drugs with pleiotropic modes of action, as is likely for miltefosine, the nature of the interaction could differ between doses. When the data obtained were ranked, combinations with the maximal tolerable drug exposure seem more relevant than combinations with suboptimal ones (18).

There are various accepted mechanisms underlying the different interactions (18).

However, the mechanisms of action of antileishmanial drugs are still poorly understood. Suggested targets of miltefosine in *Leishmania* include perturbation of ether-lipid metabolism, glycosylphosphatidylinositol anchor biosynthesis, and signal transduction (21) as well as inhibition of the glycosome-located alkyl-specific acyl coenzyme A acyltransferase, an enzyme involved in lipid remodeling (22). Recently, it has been demonstrated that miltefosine inserts into the membrane by miscibility and interacts with sterols (36). The toxicity of amphotericin B is through its binding to sterols in the cell membrane and formation of aqueous pores (4). A higher affinity for 24-substituted sterols, predominant in *Leishmania* and fungi, than for cholesterol, predominant in mammalian cell membranes, determines its selectivity, and C-24-alkylated sterols are absent from *L. donovani* promastigotes resistant to amphotericin B (35). However, a similar content of 24-alkylated sterols was found in wild-type and miltefosine-resistant promastigotes at the plasma membrane level (37). Still, the membrane could be the site of the interaction between miltefosine and amphotericin B.

Studies on the mode of action of paromomycin in *Leishmania* are sparse, with mitochondrial ribosomes (24), induction of respiratory dysfunction, and mitochondrial membrane depolarization implicated as suggested targets (23). Pentavalent antimonials are generally regarded as prodrugs requiring conversion to the trivalent form (Sb<sup>III</sup>), and suggested targets have

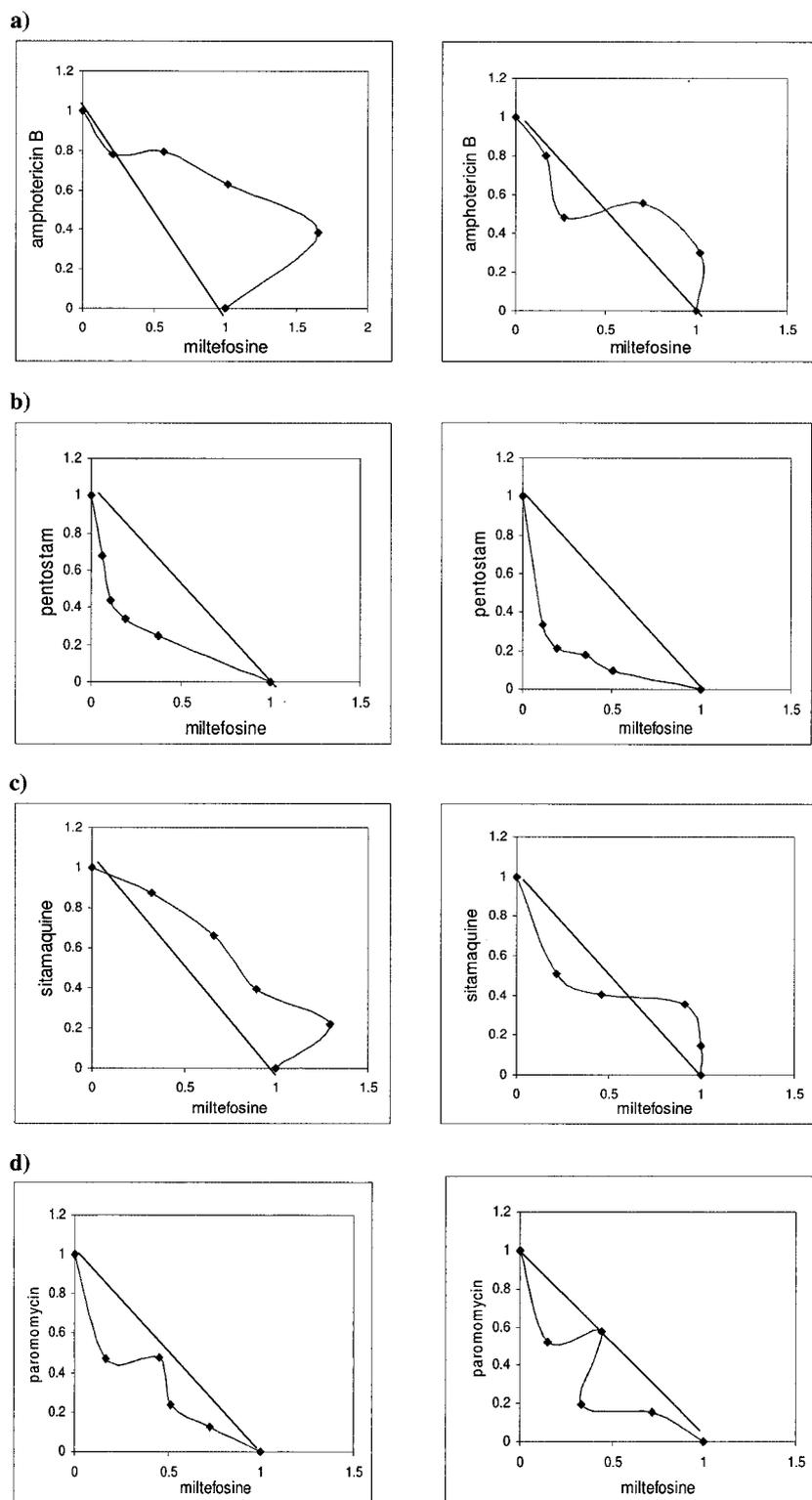


FIG. 1. Representative isobolograms of in vitro interactions against intracellular amastigotes. Interactions are given at the  $EC_{50}$  (left) and  $EC_{90}$  (right) levels. Numbers on the axes represent normalized FICs of miltefosine (x axis) and of the respective partner drugs (y axes). (a) Miltefosine-amphotericin B; (b) miltefosine-sodium stibogluconate; (c) miltefosine-sitamaquine; (d) miltefosine-paromomycin. Drugs were used in their salt forms as stated in the text.

TABLE 3. AEIs for in vivo interactions

Partner drug	Dose (mg/kg)	% Inhibition <sup>a</sup> ± SEM	ED <sub>50</sub> of miltefosine (mg/kg)	AEI
Sodium stibogluconate	0		5.06	
	3.3	2.12 ± 6.13	4.41	1.15
	10	45.37 ± 5.94	4.68	1.08
	30	79.08 ± 5.33	2.13	2.38
Amphotericin B	0		11.08	
	0.05	20.58 ± 6.26	4.01	2.76
	0.16	24.89 ± 3.54	3.27	3.39
	0.5	50.22 ± 4.51	0.98	11.31
Paromomycin	0		7.36	
	7	17.07 ± 11.7	4.52	1.63
	21	36.23 ± 5.83	11.14	0.66
	63	41.88 ± 3.67	1.02	7.22

<sup>a</sup> % Inhibition, the percent inhibition of the partner drug alone at this dose level in the respective experiment.

been glycolysis, macromolecular biosynthesis, and trypanothione (50).

In addition to mechanistic interactions, pharmacokinetic interactions can occur where absorption, distribution, or elimination of a drug is altered, modifying the efficacy of component drugs (6). "Pharmacokinetic synergism" has been shown for rifampin and quinolones, where increased activity of the combination in vivo is due to improved tissue distribution rather than in vitro synergism (13). Matching pharmacokinetics are important in combination therapy that aims at preventing the emergence of drug resistance. Elimination half-life is one factor that is important for the mutual protection of combined drugs (20). In malaria combination therapy, the time to peak concentrations has been used to explain potentiation effects between atovaquone and proguanil (38). However, given the intracellular localization of the *Leishmania* parasite in organs of the reticuloendothelial system, factors like volume of distribution, tissue distribution, and uptake into macrophages are of equal importance. In this context, it is important to compare the pharmacokinetics of the administered drugs, as these fac-

tors contribute to the interactions in vivo. The plasma half-life of amphotericin B is 89 min, and the volume of distribution was 0.998 liters/kg in mice after intravenous administration (12). The plasma half-life of miltefosine was determined to be 96 h (25), and the volume of distribution was 0.7 liters/kg after oral administration in rats (19). Sodium stibogluconate had a longer half-life (1.46 h) and a smaller volume of distribution (0.25 liters/kg) when administered intravenously to dogs (29).

Combined treatments with various drugs showing some degree of efficacy against *Leishmania* have been reported previously (7, 27), but combination therapy is not standard in the treatment of leishmaniasis. This has been due to the limited availability of effective antileishmanial drugs. With miltefosine, one other effective and oral drug is added to the armory and might change this picture. Advantages of combination therapy have to be weighed against potential disadvantages, as combinations of drugs could increase the likelihood or severity of adverse effects. This is a special concern if overlapping toxicity between drugs exists in the first place. Advantages include the delay or prevention of the development of resistance (9) and shorter treatment regimens that could improve compliance and reduce cost. Leishmaniasis is a neglected disease, and there are few drugs in development; it is essential not to jeopardize the life span of new chemical entities. Potential problems arising from the unrestricted use of miltefosine for monotherapy in India have been pointed out recently (46).

In conclusion, (i) none of the drugs tested decreased miltefosine activity in vivo, (ii) no signs of toxicity were recorded for any of the combinations tested in vivo, and (iii) the in vivo data favor a combination of miltefosine and amphotericin B or paromomycin rather than miltefosine and sodium stibogluconate. This last point might be particularly relevant to treat antimony-resistant VL cases in India. Considering both toxicity and cost of the partner drug (26), paromomycin would appear to be a better option than amphotericin B.

However, further studies to extend the matrix of interactions of antileishmanials, including liposomal formulations of amphotericin B, are under way. Ultimately, any combination will

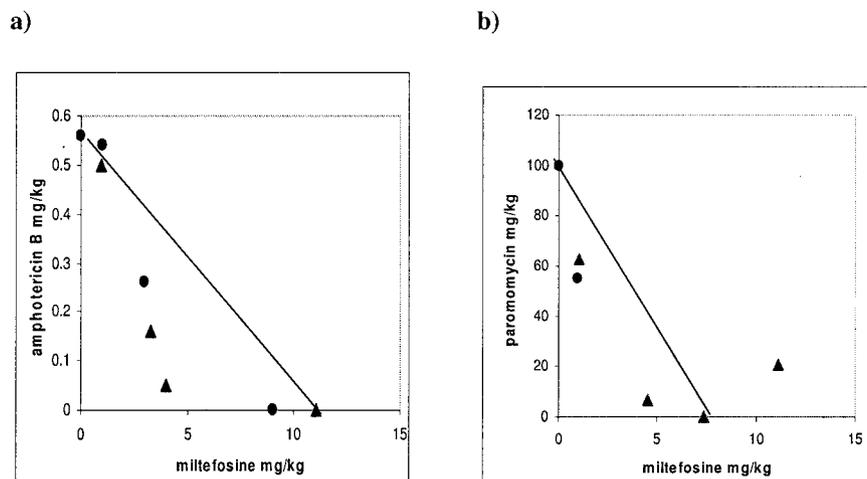


FIG. 2. Isobolograms of the in vivo interactions of (a) miltefosine-amphotericin B and (b) miltefosine-paromomycin. Triangles indicate ED<sub>50</sub>s of miltefosine on the x axis when given with the dose of amphotericin B or paromomycin indicated on the y axis. Conversely, closed circles indicate ED<sub>50</sub>s of amphotericin B or paromomycin on the y axis when given with the dose of miltefosine indicated on the x axis.

have to be tested in human patients and controlled clinical trials to prove useful for patients suffering from VL.

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