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Translational pharmacokinetic modelling and simulation for the assessment of duration of contraceptive use after treatment with miltefosine

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Objectives: Use of miltefosine in the treatment of visceral leishmaniasis (VL) is hampered by its potential teratogenicity. The duration of adequate contraceptive cover in females of child-bearing potential after cessation of a potentially teratogenic drug therapy remains debated. The objective of this study was to provide a rational approach to suggest durations of contraceptive cover for various miltefosine regimens.

Methods: A human reproductive safety threshold exposure limit was derived using animal-to-human dose conversion. Pharmacokinetic (PK) data for miltefosine in females are lacking; a previously developed population PK model and a comprehensive anthropometric dataset were used to simulate PK data for Indian female VL patients receiving miltefosine for 5, 7, 10 or 28 days. Probability of supra-threshold miltefosine exposure was used to evaluate adequate durations of post-treatment contraceptive cover for the various regimens.

Results: PK data were simulated for 465 treated Indian female VL patients of child-bearing potential with a median age of 25 years (IQR 16–31 years) and median weight of 38 kg (IQR 34–42 kg). From animal reproductive toxicity studies, a human reproductive safety threshold exposure limit was derived of 24.5 μg·day/mL. Probability of 'unprotected' supra-threshold miltefosine exposure was very low (<0.2%) for a post-treatment contraceptive cover period of 4 months for the standard 28 day regimen, and of 2 months for the shorter regimens.

Conclusions: To our knowledge, this is the first study providing rational suggestions for contraceptive cover for a teratogenic drug based on animal-to-human dose conversion. For the 28 day miltefosine regimen, post-treatment contraceptive cover may be extended to 4 months, whereas for all shorter regimens 2 months may be adequate.

Keywords: reproductive health, reproductive toxicity, contraception, anticonceptive, teratogenicity, pharmacokinetics, population pharmacokinetics, visceral leishmaniasis, leishmaniasis, Monte Carlo simulation, translational research, pharmacometrics

Introduction

Miltefosine is currently the only oral drug available for the treatment of visceral leishmaniasis (VL), a neglected tropical infection caused by unicellular parasites. The drug has been rolled out as first-line treatment for VL in India (28 day regimen, 2.5 mg/kg/day) and has been adopted in several national VL elimination programmes (e.g. in India, Bangladesh and Nepal).[1,2] One of the most important factors hampering clinical use of miltefosine, certainly in rural areas, is its potential reproductive toxicity, which was demonstrated in animal studies but remains to be confirmed in humans. Feto- and embryotoxicity were demonstrated in both rabbits and rats, while teratogenicity was shown only in rats, with the first drug-induced deformities noticed at a dosage of 1.2 mg/kg given for a period of 10 days during gestation.[3] As a consequence, the current guidelines for the use of miltefosine

strictly prohibit its use during pregnancy and recommend, for women of child-bearing potential, a period of contraceptive cover of 3 months after cessation of therapy following the standard 28 day miltefosine regimen, based on a simple extrapolation of the elimination half-life of miltefosine (7 days).[3–5] In some of the literature and guidelines a period of just 2 months is being recommended.[6,7] Previously, we showed that miltefosine has an extremely long terminal elimination half-life of 31 days, and could be detected in blood plasma at least 5 months after the end of therapy (150 mg/day for 28 days).[8] Since the teratogenic effect level for miltefosine remains unknown in humans, based on these data the contraceptive cover could also arbitrarily be extended to at least 5 months post-treatment.[8]

Recent studies in India have shown that the original 28 day miltefosine treatment regimen can be shortened if combined with liposomal amphotericin B.[9,10] Currently, new studies on combination therapies for VL are being designed and conducted with various shortened miltefosine regimens.[11,12] The inclusion of women of child-bearing potential is being considered, but the length and type of contraceptive coverage remain important issues and constitute points of discussion. Finding the optimal contraceptive coverage touches upon an important ethical dilemma as well: too long a period of contraceptive cover may be economically unfavourable and cause concerns for adherence (e.g. barrier or oral contraception), while too short a period may increase the risk of congenital malformation. Therefore, more knowledge is urgently needed on the expected miltefosine levels in women of child-bearing potential from areas where VL is endemic, to support a more rational risk management strategy against the teratogenic potential of miltefosine. Unfortunately, most of the previous controlled clinical studies with miltefosine excluded women of child-bearing potential, and pharmacokinetic data are not available for this population. This study aimed at providing a more scientific and rational approach to suggest durations of contraceptive cover after the use of miltefosine. It was based on conversion and translation of dosing data from preclinical reproductive toxicity studies in animals and on simulations of human pharmacokinetic data using a comprehensive anthropometric dataset of an historic cohort of Indian VL patients.

Methods

Anthropometric data

Anthropometric data for VL patients were derived from a large demographic dataset from Médecins Sans Frontières-Operational Centre Barcelona-Athens (MSF-OCBA), which was collected between 2007 and 2009 from Hajipur SADR Hospital in Vaishali District, Bihar State, India.

A group of typical Indian female VL patients of child-bearing potential was selected from this dataset based on the following criteria: sex (female) and child-bearing age (12–45 years, inclusive). All individual records lacking either weight or height data were removed from this anthropometric dataset, because both values are needed to estimate fat-free body mass (FFM).

FFM in kg was estimated from total body weight (WT) in kg and height (HT) in m as follows:[13]

$$\mathrm{FFM} = \mathrm{WHS}_{\max} \times \mathrm{HT}^2 \times \left(\frac{\mathrm{WT}}{\mathrm{WHS}_{50} \times \mathrm{HT}^2 + \mathrm{WT}}\right) \quad (1)$$

where, for females, $\mathrm{WHS}_{\max}$ (maximum weight for height standard) is 37.99 kg/m^2 and WHS_{50} (half maximal weight for height standard) is 35.98 kg/m^2.[13]

Simulations using a population pharmacokinetic model

All calculations, simulations and estimations were performed on a dual-core desktop computer running NONMEM 7.2,[14] the R statistical software package (version 2.14.0; http://www.r-project.org/)[15] and Perl speaks NONMEM (PsN, version 3.4.2; http://psn.sourceforge.net).[16] Piraña (version 2.3; an interface to NONMEM, PsN, and our cluster; http://www.pirana-software.com) was used for run deployment and analysis.[17]

Monte Carlo simulations (simulations based on repeated random sampling for individuals) using a non-linear mixed-effects model were performed with NONMEM, and the output from NONMEM was processed, interpreted and visualized with R. Prediction plots were also made using R. An open two-compartment model was used for the simulations, with first-order absorption and elimination from the central compartment; parameter estimates were validated previously with miltefosine pharmacokinetic data from Indian individuals.[54] To account for the effects of body size on the pharmacokinetics of miltefosine, allometric scaling of clearance and volume of distribution by fat-free mass was applied, since this was previously validated as the best body size model and descriptor for miltefosine over a wide range of body size. For example, for drug clearance the following equation was used:

$$\mathrm{CL}/F_i = \theta_1 \times \left(\frac{\mathrm{FFM}_i}{\mathrm{FFM}_{std}}\right)^{\mathrm{PWR}} \times \exp(\eta_{i,\mathrm{CL}/F}) \quad (2)$$

where CL/F_i represents the clearance of the ith individual; θ_1 is the typical value of clearance and $\eta_{i,\mathrm{CL}/F}$ is the between-subject random effect with a mean of 0 and a variance of ω^2; FFM_i is the calculated fat-free body mass of the ith individual (see Equation 1); $\mathrm{FFM}_{\mathrm{std}}$ is a standard fat-free body mass (set at 53 kg, because pharmacokinetic parameters were normalized to this value); and *PWR* is the allometric power exponent, which was fixed for clearance at 0.75 and for volume of distribution at 1.0, based on biological principles that support these values.[18–21] Parameters that were previously estimated were fixed in the simulations and are summarized in Table 1. Bioavailability (F) was unknown, and therefore, parameters relative to bioavailability were used (CL/F, V/F, etc.).

Simulated miltefosine dose regimens

Several durations of miltefosine treatment were simulated individually (as described above) for the selected Indian female VL patients of child-bearing potential. These Monte Carlo random pharmacokinetic simulations were repeated 100 times. The absolute daily dose (mg/day) was similar between these regimens, and conformed to the current guidelines for miltefosine usage in India: individuals with a body weight <25 kg received 50 mg of miltefosine once daily, while individuals with a body weight ≥ 25 kg received 50 mg twice daily with a 12 hour interval (total, 100 mg/day).[7] Treatment durations of 5, 7, 10 and 28 days were separately simulated.

Conversion of drug dose from animal to human and definition of a reproductive safety threshold exposure limit

The no observed adverse effect level (NOAEL) of miltefosine (specifically, the level of exposure at which no reproductive toxicity was observed in the most sensitive animal test species, rat) was determined from previous preclinical teratogenicity, feto- and embryotoxicity studies. This NOAEL was translated to a total human equivalent dosage using the

dose calculator tool available from the FDA.[22] In brief, the total dose per body weight in rats (mg/kg) was converted to total dose per body surface area (BSA; mg/m^2) using a default rat body weight (WT_{rat}) of 0.15 kg and BSA of 0.025 m^2.[23,24] This dose per BSA was converted to a total NOAEL human equivalent dose using Boyd's formula for BSA,[25] as shown in Equation 3:

$$HED = \frac{Dose_{rat} \times WT_{rat}}{BSA_{rat}} \times \left(0.0003207 \times WT_{human}^{(0.7285-0.0188\log_{10} WT_{human})} \times HT_{human}^{0.3}\right) \quad (3)$$

Where HED is the total human equivalent dose and WT_{human} and HT_{human} are the median weight (g) and height (cm), respectively, of Indian female VL patients of child-bearing potential in the anthropometric database (MSF-OCBA).

Miltefosine drug exposure [area under the curve from zero to infinity ($AUC_{0-\infty}$)] following administration of the total NOAEL human equivalent dose in a population of Indian females of child-bearing potential was simulated as described above. Based on these simulations, a reproductive safety threshold exposure limit for miltefosine in humans was defined as the median miltefosine exposure following administration of the total NOAEL human equivalent dose. To account for any unknown difference in sensitivity to the reproductive toxicity of miltefosine between humans and animal test species, the reproductive safety threshold exposure limit was divided by a default animal-to-human uncertainty factor of 10.[26–30]

Table 1. Final population pharmacokinetic parameter estimates

Parameter	Estimate	Between-subject variability (%)	Parameter estimate uncertainty [relative SE (%)]
Absorption rate (k_a) (h^{-1})	0.416	18.2	11.5
Clearance (CL/*F*) (L/day)	3.99	32.1[a]	3.5
Volume of central compartment (V_2/*F*) (L)	40.1	34.1[a]	4.5
Intercompartmental clearance (Q/*F*) (L/day)	0.0347	not estimated	18.3
Volume of peripheral compartment (V_3/*F*) (L)	1.75	not estimated	8.2
Residual variability (%)	34.3	not estimated	3.7

Final estimates from the miltefosine population pharmacokinetic model with two compartments and allometric scaling by fat-free mass. CL/*F* and *V*/*F* are normalized to a fat-free mass of 53 kg and scaled allometrically for CL/*F* with a power of 0.75 for CL/*F* and 1 for *V*/*F*.[54]
[a]Between-subject variabilities in CL/*F* and V_2/*F* correlated with a correlation coefficient of 0.92.

Probability of exposure above the reproductive safety threshold exposure limit

The 'unprotected' residual exposure to miltefosine after end of the contraceptive cover period until infinity (depicted schematically in Figure 1) was determined with NONMEM in the individual simulated pharmacokinetic curves for the selected Indian female VL patients of child-bearing potential, as described above, for the different miltefosine treatment durations under consideration (5, 7, 10 or 28 days). Different periods of contraceptive cover were considered: 1, 2, 3 and 4 months of contraceptive use after the end of treatment. For example, for the

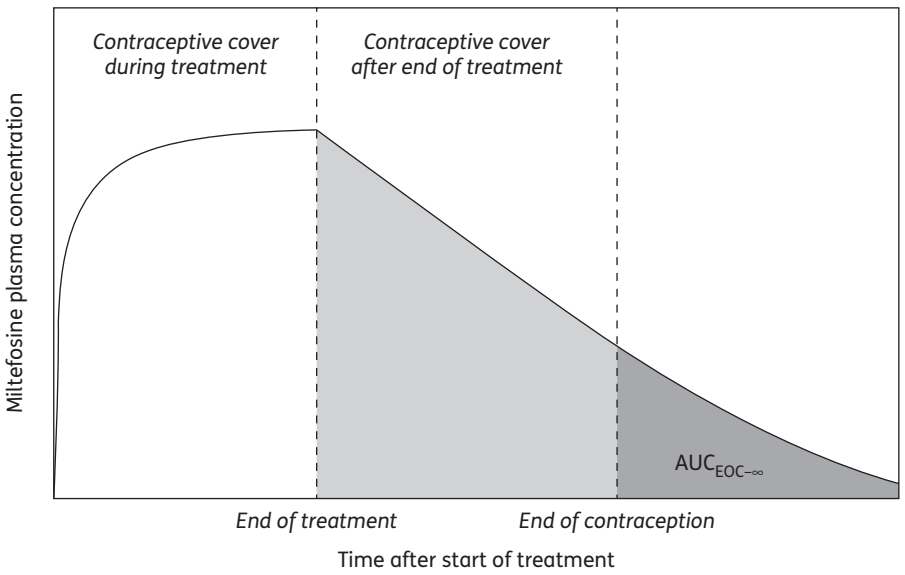

Figure 1. Schematic depiction of contraceptive cover in relation to miltefosine exposure. The figure shows a typical curve of miltefosine plasma concentration versus time. Contraceptive cover is required during treatment and for an extra (variable) period after the end of treatment (indicated between the broken lines). Miltefosine exposure is indicated during treatment and with contraceptive cover (white), after treatment but with contraceptive cover (light grey) and after end of contraception (EOC; dark grey). In this study we focused on miltefosine $AUC_{EOC-\infty}$ for different durations of miltefosine treatment and different durations of the contraceptive cover period.

28 day miltefosine regimen and a 3 month contraception period the AUC was calculated from end of contraception (EOC), which is day 118 after start of treatment, until infinity ($AUC_{EOC-\infty}$; Figure 1). Cumulative $AUC_{EOC-\infty}$ values were calculated in NONMEM by integrating the amounts in dummy compartments, according to Equation 4:

$$AUC_{EOC-\infty} = \int_{EOC}^{\infty} C_t \cdot dt \quad (4)$$

Each individual $AUC_{EOC-\infty}$ was compared with the reproductive safety threshold exposure limit. The probability of Indian female VL patients of child-bearing potential having exposure exceeding the threshold exposure limit in the simulations was calculated for all four different treatment durations and lengths of post-treatment contraceptive cover.

Results

Anthropometric data

The anthropometric dataset of VL patients from Bihar provided by MSF-OCBA contained 2264 individuals of which 465 were eligible females of child-bearing potential (12–45 years, inclusive). The most salient demographic characteristics of the selected female VL patients are depicted in Table 2.

Population pharmacokinetic Monte Carlo simulations

Of the 465 selected women, 21 (4.5%) had a body weight <25 kg and were allocated a miltefosine dosage of 50 mg once daily in the simulations, while the other women were allocated a dosage of 50 mg twice daily, according to the standard miltefosine treatment guidelines in India.[7] The mean daily miltefosine dosage per kg of body weight was 2.37 mg/kg (2.08–2.63 mg/kg) for body weights <25 kg, and 2.67 mg/kg (1.42–4.00 mg/kg) for body weights ≥25 kg.

For each individual, a pharmacokinetic curve was simulated for miltefosine for each of the four different treatment lengths (5, 7, 10 and 28 days). Figure 2 depict the median concentrations during and after treatment and the 90% prediction interval for the respective dose regimens, extracted from these simulations ($n = 465 \times 100$). Simulated miltefosine plasma concentrations at various timepoints after start of treatment were evaluated and are shown in Table 3. Additionally, the median times (90% prediction interval) until the simulated miltefosine plasma concentration curves reached the lower limit of quantification [of the most sensitive available detection method for miltefosine in human plasma (4 ng/mL)][31] for the 5, 7, 10 and 28 day regimens were 158 days (103–216 days), 176 days (119–235 days), 196 days (139–255 days) and 258 days (201–318 days), respectively.

Table 2. Demographic characteristics of selected female Indian VL patients of child-bearing potential ($n = 465$)

Parameter	Median value (IQR)
Age (years)	25 (16–31)
Weight (kg)	38 (34–42)
Height (cm)	148 (144–152)
Body mass index (kg/m^2)	17.3 (15.8–18.8)
Fat-free body mass (kg)	27.1 (24.6–29.5)

Conversion of a reproductive safety threshold exposure limit and translating drug exposure in animal to human

In reproductive animal studies, oral doses ≥1.2 mg/kg/day given for 10 days to pregnant rats led to teratogenicity, so the maximum miltefosine dose that led to the NOAEL was therefore 0.6 mg/kg/day.[3,32,33] Given the pharmacokinetic properties of miltefosine (extremely long primary and terminal elimination half-life, and thus a high accumulation of dosages in terms of total exposure), a single total dose of miltefosine was regarded as equivalent to the same total dosage spread over a number of days (e.g. 10 days). Therefore, in rat (body weight 0.15 kg, BSA 0.025 m^2), a repeated miltefosine dose of 0.6 mg/kg/day for 10 days corresponds to a total dose of 0.9 mg or 36 mg/m^2. Converting this NOAEL BSA-normalized dose to a human equivalent dose (body weight 38 kg, height 148 cm), a 36 mg/m^2 dose in rat results in a total single human equivalent dose of 45 mg (50 mg, to the nearest miltefosine capsule unit). The median $AUC_{0-\infty}$ (90% prediction interval) following administration of 50 mg in the selected Indian female VL patients ($n = 465$) was 245 μg·day/mL (range, 140–467 μg·day/mL). When divided by an animal-to-human uncertainty factor of 10, the reproductive safety threshold exposure limit was determined to be 24.5 μg·day/mL.

Probability of exposure above the reproductive safety threshold exposure limit

Using the simulated individual pharmacokinetic curves for different miltefosine regimens (5, 7, 10 and 28 days) as described above, the miltefosine exposures after the end of contraceptive cover ($AUC_{EOC-\infty}$) were analysed (Table 4 and Figure 1). The probability of an Indian female VL patient having post-contraceptive (unprotected) exposure to miltefosine above the identified reproductive safety threshold exposure limit in the simulations is shown in Table 5. A 1 month period of contraceptive cover after end of treatment led to unprotected exposure to miltefosine exceeding the threshold exposure limit in a proportion of simulated females in all treatment regimens. For the 5, 7 and 10 day regimens, 2 months of contraceptive cover after cessation of treatment were sufficient to reduce the probability of having a supra-threshold miltefosine exposure to <0.2%, while for the 28 day regimen, 4 months of contraceptive cover was needed to reach <0.2% probability (Table 5).

Discussion

To our knowledge, this is the first study that provides suggestions for contraceptive cover for a potentially teratogenic drug, based on dose conversion from preclinical teratogenicity studies to humans. A reproductive safety threshold exposure limit was defined for miltefosine based on the conversion of the NOAEL dose in animal reproductive toxicity studies to humans. Miltefosine exposure was simulated for Indian female VL patients of child-bearing potential following treatment with different

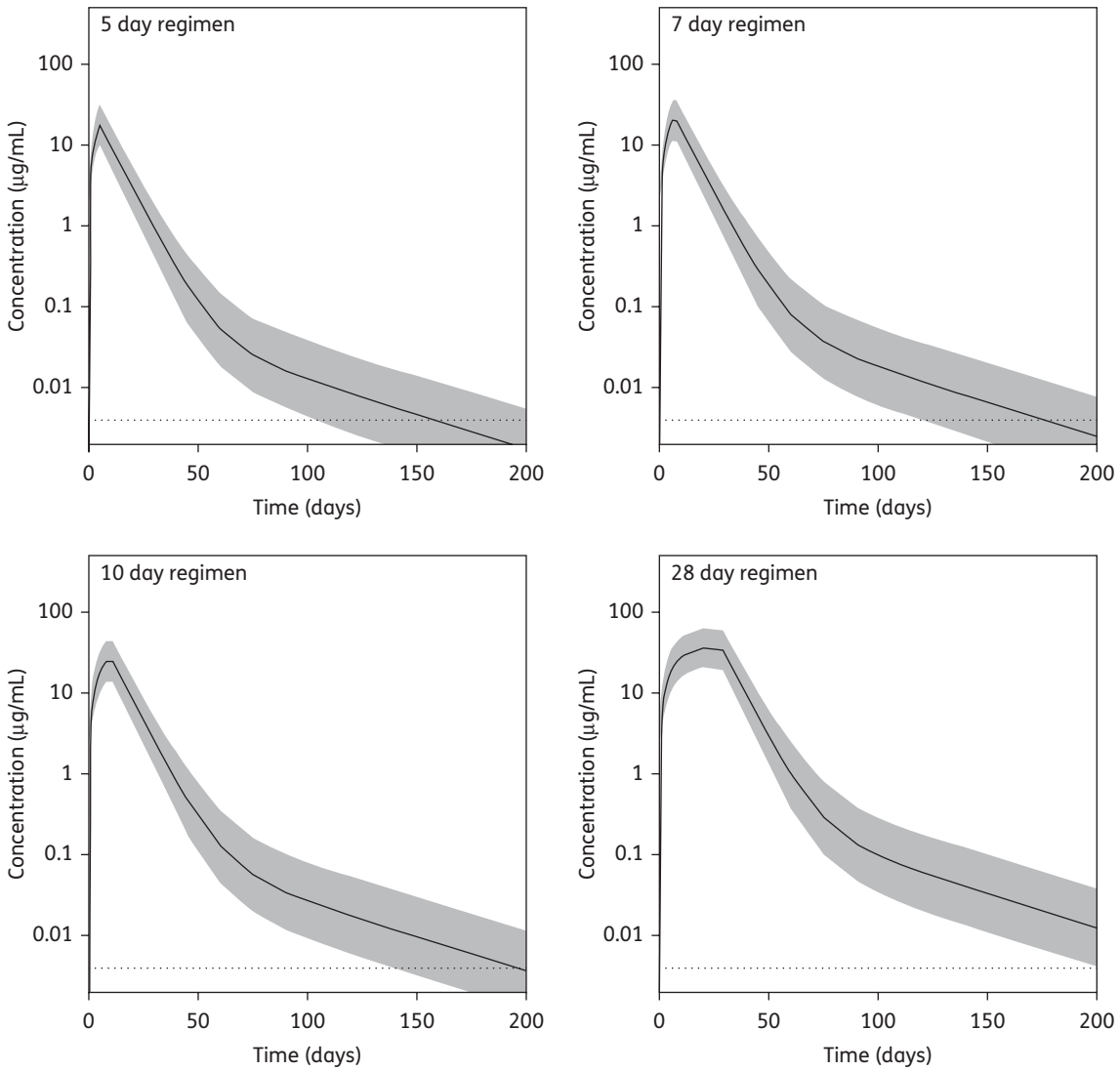

Figure 2. Monte Carlo simulations of miltefosine concentration–time curves for various dosing regimens. Median predicted miltefosine plasma concentrations (black line) and 90% prediction intervals (grey area) after different miltefosine dosing regimens (5, 7, 10 and 28 days) in Indian female VL patients of child-bearing potential (12–45 years). The broken lines indicate the current lower limit of quantification of miltefosine in plasma (4 ng/mL).[31]

miltefosine regimens, by making use of a large comprehensive dataset of anthropometric data for Indian VL patients and population pharmacokinetic Monte Carlo simulations. Probability analysis of supra-threshold exposure to miltefosine suggested a period of contraceptive cover after cessation of treatment of 2 months for a 5, 7 or 10 day miltefosine regimen and 4 months for a standard 28 day regimen.

The design of teratogenic risk-management programmes and strategies for drugs exhibiting reproductive toxicity in preclinical studies is problematic, often lacking rational consideration and usually not taking account of data from preclinical studies. Human maternal or fetal pharmacokinetic data, which could facilitate the estimation of minimum human teratogenic effect levels of these drugs, are very rarely available. Even animal pharmacokinetic data from reproductive toxicity studies are usually lacking, or at least not publicly available, thus complicating extrapolation of the teratogenic dose–effect relationship.[34] Theoretical physiological or pharmacokinetic considerations are sometimes included in these risk-management strategies. For ribavirin, an antiviral drug used in the treatment of hepatitis C infections, a 6 month period of contraceptive cover after cessation of therapy is recommended, based on the turnover time of erythrocytes, in which the drug tends to accumulate.[35,36] For isotretinoin, a vitamin A derivative used in the treatment of cystic acne vulgaris, the time until retinoic acid levels have returned to normal (endogenous) after end of treatment is taken to define the reproductive safety period.[37–40] Another related approach is based on the time until the drug becomes undetectable in plasma (e.g. for leflunomide, a pyrimidine synthesis inhibitor in the treatment of rheumatoid arthritis).[41–43]

Table 3. Simulated miltefosine concentrations in Indian female VL patients of child-bearing potential at various timepoints (EOT and at 30, 60, 90 and 180 days after start of treatment)

Miltefosine regimen	Miltefosine concentrations (ng/mL), median (90% PI)				
	EOT	day 30[a]	day 60[a]	day 90[a]	day 180[a]
5 day	16000 (6200–32000)	850 (260–2100)	51 (13–160)	15 (4.3–51)	2.5 (0.69–8.6)
7 day	19000 (7600–42000)	1400 (430–3400)	79 (22–230)	21 (4.8–69)	3.4 (0.75–12)
10 day	24000 (9900–49000)	2400 (870–5800)	130 (34–450)	34 (9.8–120)	5.5 (1.3–19)
28 day	30000 (11000–63000)	27000 (8000–60000)	940 (300–3000)	120 (33–410)	17 (4.6–65)

EOT, end of treatment; PI, prediction interval.
[a]Time in days after start of treatment.

Table 4. Exposure to miltefosine ($AUC_{EOC-\infty}$) after different miltefosine regimens (5, 7, 10 or 28 days) simulated in typical Indian female VL patients of child-bearing potential

Miltefosine regimen	No. of months on contraception	$AUC_{EOC-\infty}$ (μg·day/mL), median (90% PI)
5 days	1 month	9.97 (3.95–23.10)
	2 months	1.65 (0.58–4.68)
	3 months	0.78 (0.26–2.35)
7 days	1 month	15.42 (6.19–35.42)
	2 months	2.38 (0.83–6.82)
	3 months	1.11 (0.37–3.38)
10 days	1 month	26.02 (10.85–58.90)
	2 months	3.62 (1.28–10.36)
	3 months	1.64 (0.55–5.03)
28 days	1 month	54.50 (22.92–125.74)
	2 months	8.74 (3.08–25.19)
	3 months	4.11 (1.37–12.52)

PI, prediction interval.

Table 5. Probability of having an exposure to miltefosine after EOC exceeding the reproductive toxicity safety threshold exposure limit (24.5 μg·day/mL) for four different miltefosine treatment durations, and for different lengths of contraceptive cover

Miltefosine regimen	Probability of exposure above the reproductive safety threshold exposure limit for the indicated number of months on contraception after EOT			
	1 month	2 months	3 months	4 months
5 days	**4.3%**	<0.1%	<0.1%	<0.1%
7 days	**18.2%**	<0.1%	<0.1%	<0.1%
10 days	**54.6%**	0.198%	<0.1%	<0.1%
28 days	**93.6%**	**5.42%**	**0.581%**	<0.1%

EOT, end of therapy.
Figures in bold type indicate probabilities above the acceptable upper limit of probability for supra-threshold miltefosine exposure, which was set at one-tenth of the overall congenital malformation rate (i.e. at 0.244%).

Several disadvantages are associated with the latter approach. Firstly, with the increasing sensitivity of analytical techniques and equipment, the time until the drug becomes undetectable will increase, and thus recommendations based on this approach are likely to change over time. Secondly, the bioanalytical lower limit of quantification in plasma is not necessarily related to any teratogenic concentration–effect relationship, and it does not take into account relative drug accumulation in the uterus or fetus, making this approach rather arbitrary in relation to the actual risk involved.

Approaches for the definition of reproductive safety periods incorporating data from preclinical reproductive toxicity studies in animals and making use of translational pharmacokinetic modelling and simulation have rarely been reported. Physiologically based pharmacokinetic (PBPK) modelling has been applied in reproductive toxicology in both animals and humans to predict properties such as fetal exposure and lactational transfer, but has rarely been used in the development of teratogenic risk-management strategies for drugs.[44–46] In the current study, the NOAEL dose in animal reproductive studies was used to determine a reproductive safety threshold exposure limit for miltefosine in humans. Modelling and simulation (M&S) allowed us to assess non-parametric probability estimations in the population, taking into account the full variability profile of the pharmacokinetics of miltefosine. Simple extrapolation of the point estimates of drug-elimination half-life does not account for these probability estimations, and plausibly leads to underestimation of probability and thus associated risks. The M&S technique that was demonstrated here, therefore, provides a more rational approach to suggest a contraceptive cover period after cessation of therapy. The suggestions following from this analysis might be instrumental in deciding the length and type of contraceptive cover to be specified in miltefosine treatment guidelines. Nevertheless, these suggestions should also be interpreted with caution because of some important study assumptions and limitations.

Firstly, determination of the maximum safe miltefosine dose in pregnant rats was previously performed in a small set of animals, and so should be regarded as presumptive evidence—a general limitation of preclinical reproductive toxicity studies. On the other hand, dose administration in teratogenicity studies was performed for an extended critical period during gestation, taking into account worst-case scenarios.[3] Additionally, a default well-supported animal-to-human uncertainty factor of 10 was applied.[24,29,30]

Secondly, it must be considered that animal dose regimens remain difficult to extrapolate to humans without further data on miltefosine pharmacokinetics for both pregnant animals and humans, although there is no current evidence that miltefosine distribution and metabolism, mainly through phospholipases, is significantly different in animal species compared with humans.[3,47] Nevertheless, the probability analysis presented here would have improved significantly if more preclinical data on fetal or maternal drug levels of miltefosine in the reproductive toxicity studies in animals had been available to incorporate into a PBPK model. This would have allowed the extrapolation of a concentration–effect relationship, rather than the more indirect and less accurate dose–effect relationship that was applied here. It might therefore be recommended to emphasize the need for additional pharmacokinetic data collection in reproductive studies in animals, for the specific purpose of regulatory guidelines.[48]

Thirdly, the pharmacokinetic model that we employed in this study was previously estimated from data from European adult males, Indian adult males and Indian children.[8,54] Until now no data have been collected on miltefosine pharmacokinetics in females. Again, based on the known pathways of miltefosine metabolism, differences in population pharmacokinetic model parameter estimates between males and females are not expected. Evaluation of pharmacokinetics should be prioritized during drug development for neglected tropical diseases, particularly in rare and vulnerable populations, to help rationalize and optimize both dose regimens and informed clinical risk management.[6]

It remains complicated to define the adequacy of contraceptive cover periods based on the calculated probability of having a post-contraceptive exposure above the identified reproductive safety threshold exposure limit. The general incidence rate for congenital malformations or anomalies should to be taken into account as well, since this depends on various (unknown) cumulative genetic and environmental risk factors. In India, the overall incidence of congenital malformation appeared to range between 0.3% and 3.6%.[49,50] In Europe, a more accurate overall incidence of 2.44% was reported,[51] which may be explained by a higher autopsy rate in the included European centres. Around 10% of congenital malformations are environmentally induced.[52] To define contraceptive adequacy, it might therefore be appropriate to set the acceptable upper limit of probability for supra-threshold miltefosine exposure at one-tenth of the overall congenital malformation rate (i.e. at 0.244%). With this assumption, these findings would support adequate contraceptive cover after cessation of treatment of 2 months for a 5, 7 and 10 day miltefosine regimen, and 4 months for the standard 28 day miltefosine regimen (Table 5). Most notably, this suggested post-treatment contraceptive cover period for the 28 day regimen is longer than the currently advised period of 2 or 3 months.[3–7]

An additional important factor that needs to be taken into account is the type of contraceptive cover. Methods such as barrier contraception and the oral contraceptive pill may not be adequate due to low compliance or diminished efficacy (e.g. due to vomiting resulting from miltefosine use). Other forms of contraception, such as implants, intra-uterine devices or sterilization may be unnecessarily long-term for the period of cover required. Depot contraceptives (e.g. medroxyprogesterone acetate) may provide adequate coverage (3 months) for at least the 5, 7 or 10 day regimen, but may not adequately remove risk for the 28 day regimen. Moreover, all these methods need to be reviewed in the context of what is culturally appropriate, recommended or locally available.

Contraceptive recommendations and pregnancy precautions are currently only given to female patients and not to male patients receiving miltefosine. Although preclinical animal studies did show (reversible) testicular atrophy and impaired fertility in male rats at a dose of 8.25 mg/kg,[3] spermiogram analyses in Colombian male patients as well as limited retrospective analyses of reproductive performance in Indian male patients suggested absence of any clinically relevant effect on male fertility.[7] Conversely, recently it was shown in a retrospective observational study that a large proportion of miltefosine-treated males experienced a substantial treatment-related reversible reduction in ejaculate.[53] Although nothing is known about sperm count and quality in these patients, this finding clearly points to effects of miltefosine on the male reproductive system. In order to fully evaluate the appropriateness of recommending additional male contraceptive measures, such as barrier protection and counselling during and after miltefosine treatment, more data are needed on the mechanisms of male-mediated reproductive toxicity of miltefosine in animal studies, and semen DNA quality during and after treatment should be better evaluated.

In conclusion, we here provide suggestions for contraceptive cover periods and associated risks of drug exposure after cessation of therapy for females of child-bearing potential treated with different miltefosine regimens, based on translation of the minimum safe dose in reproductive toxicity studies in animals to a reproductive safety threshold exposure limit in humans. For the standard 28 day miltefosine regimen, the duration of post-treatment contraceptive cover may be extended to 4 months. The periods that we suggest take into account worst-case scenarios and might support a more rational teratogenic risk-management strategy for miltefosine than currently is the case.

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None to declare.

References

1 Mondal D, Singh SP, Kumar N *et al*. Visceral leishmaniasis elimination programme in India, Bangladesh, and Nepal: reshaping the case finding/case management strategy. *PLoS Negl Trop Dis* 2009; **3**: e355.

2 Sundar S, Mondal D, Rijal S *et al*. Implementation research to support the initiative on the elimination of kala azar from Bangladesh, India and Nepal–the challenges for diagnosis and treatment. *Trop Med Int Health* 2008; **13**: 2–5.

3 Sindermann H, Engel J. Development of miltefosine as an oral treatment for leishmaniasis. *Trans R Soc Trop Med Hyg* 2006; **100**: S17–20.

4 German Drug Registration Authorities. *Impavido 10/50 mg Kapseln-Fachinformation*. 2008. http://www.pharmnet-bund.de/dynamic/de/index.html (15 February 2010, date last accessed).

5 WHO. *WHO Technical Report Series 949: Control of the leishmaniases. Report of a meeting of the WHO Expert Committee on the Control of Leishmaniases, Geneva 22–26 March 2010*. 2011. http://whqlibdoc.who.int/trs/WHO_TRS_949_eng.pdf (16 January 2012, date last accessed).

6 Sundar S, Olliaro PL. Miltefosine in the treatment of leishmaniasis: Clinical evidence for informed clinical risk management. *Ther Clin Risk Manag* 2007; **3**: 733–40.

7 Government of India: Dte. of National Vector Borne Disease Control Progamme. *Guideline on Use of Miltefosine*. http://nvbdcp.gov.in/Doc/Guidelines%20on%20miltefosine.pdf (12 December 2011, date last accessed).

8 Dorlo TP, van Thiel PP, Huitema AD *et al*. Pharmacokinetics of miltefosine in Old World cutaneous leishmaniasis patients. *Antimicrob Agents Chemother* 2008; **52**: 2855–60.

9 Sundar S, Sinha PK, Verma DK *et al*. Ambisome plus miltefosine for Indian patients with kala-azar. *Trans R Soc Trop Med Hyg* 2011; **105**: 115–7.

10 Sundar S, Sinha PK, Rai M *et al*. Comparison of short-course multidrug treatment with standard therapy for visceral leishmaniasis in India: an open-label, non-inferiority, randomised controlled trial. *Lancet* 2011; **377**: 477–86.

11 Omollo R, Alexander N, Edwards T *et al*. Safety and efficacy of miltefosine alone and in combination with sodium stibogluconate and liposomal amphotericin B for the treatment of primary visceral leishmaniasis in East Africa: study protocol for a randomized controlled trial. *Trials* 2011; **12**: 166.

12 ClinicalTrials.gov. *Phase III, Study of Three Short Course Combination Regimens (Ambisome®, Miltefosine, Paromomycin) Compared With AmBisome® Alone for the Treatment of Visceral Leishmaniasis in Bangladesh*. 2011. http://clinicaltrials.gov/ct2/show/NCT01122771 (16 January 2012, date last accessed).

13 Janmahasatian S, Duffull SB, Ash S *et al*. Quantification of lean bodyweight. *Clin Pharmacokinet* 2005; **44**: 1051–65.

14 Beal SL, Boeckmann AJ, Sheiner LB. *NONMEM Users Guides*. Ellicott City, MD, USA: Icon Development Solutions, 2006.

15 R Development Core Team. *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing, 2011.

16 Lindbom L, Ribbing J, Jonsson EN. Perl-speaks-NONMEM (PsN)–a Perl module for NONMEM related programming. *Comput Methods Programs Biomed* 2004; **75**: 85–94.

17 Keizer RJ, van Benten M, Beijnen JH *et al*. Piraña and PCluster: a modeling environment and cluster infrastructure for NONMEM. *Comput Methods Programs Biomed* 2011; **101**: 72–9.

18 Holford NH. A size standard for pharmacokinetics. *Clin Pharmacokinet* 1996; **30**: 329–32.

19 Anderson BJ, Holford NH. Mechanism-based concepts of size and maturity in pharmacokinetics. *Annu Rev Pharmacol Toxicol* 2008; **48**: 303–32.

20 Anderson BJ, McKee AD, Holford NH. Size, myths and the clinical pharmacokinetics of analgesia in paediatric patients. *Clin Pharmacokinet* 1997; **33**: 313–27.

21 West GB, Brown JH, Enquist BJ. A general model for the origin of allometric scaling laws in biology. *Science* 1997; **276**: 122–6.

22 FDA. *Oncology Tools: Dose Calculator*. http://www.accessdata.fda.gov/scripts/cder/onctools/animalquery.cfm (9 September 2011, date last accessed).

23 Freireich EJ, Gehan EA, Rall DP *et al*. Quantitative comparison of toxicity of anticancer agents in mouse, rat, hamster, dog, monkey, and man. *Cancer Chemother Rep* 1966; **50**: 219–44.

24 Reagan-Shaw S, Nihal M, Ahmad N. Dose translation from animal to human studies revisited. *FASEB J* 2008; **22**: 659–61.

25 Boyd E. *The Growth of the Surface Area of the Human Body*. Minneapolis: University of Minnesota Press, 1935.

26 Dourson ML, Stara JF. Regulatory history and experimental support of uncertainty (safety) factors. *Regul Toxicol Pharmacol* 1983; **3**: 224–38.

27 Dourson ML, Felter SP, Robinson D. Evolution of science-based uncertainty factors in noncancer risk assessment. *Regul Toxicol Pharmacol* 1996; **24**: 108–20.

28 Rhomberg LR, Lewandowski TA. Methods for identifying a default cross-species scaling factor. *Hum Ecol Risk Assess* 2006; **12**: 1094–127.

29 FDA. *Guidance for Industry: Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers*. 2005. http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm078932.pdf (16 January 2012, date last accessed).

30 U.S. Environmental Protection Agency (EPA). *Guidelines for Developmental Toxicity Risk Assessment*. 1991. http://www.epa.gov/raf/publications/pdfs/DEVTOX.PDF (16 January 2012, date last accessed).

31 Dorlo TP, Hillebrand MJ, Rosing H *et al*. Development and validation of a quantitative assay for the measurement of miltefosine in human plasma by liquid chromatography–tandem mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci* 2008; **865**: 55–62.

32 Berman J. Miltefosine to treat leishmaniasis. *Expert Opin Pharmacother* 2005; **6**: 1381–8.

33 Paladin Labs Inc. *Application for Inclusion of Miltefosine on WHO Model List of Essential Medicines*. 2010. http://www.who.int/selection_medicines/committees/expert/18/applications/Miltefosine_application.pdf (13 December 2011, date last accessed).

34 Polifka JE, Friedman JM. Clinical teratology: identifying teratogenic risks in humans. *Clin Genet* 1999; **56**: 409–20.

35 Schering-Plough Research Institute. *Product Information: Rebetron™ Combination Therapy containing Rebetol® (ribavirin, USP) Capsules and Intron® A (interferon alfa-2b, recombinant) Injection*. 2001. http://www.accessdata.fda.gov/drugsatfda_docs/label/2001/20903s13lbl.pdf (9 December 2011, date last accessed).

36 Lertora JJ, Rege AB, Lacour JT *et al*. Pharmacokinetics and long-term tolerance to ribavirin in asymptomatic patients infected with human immunodeficiency virus. *Clin Pharmacol Ther* 1991; **50**: 442–9.

37 European Medicines Agency (EMA) - Committee for Proprietary Medicinal Products (CPMP). *Roaccutane - Article 30 referral - Annex I, II, III*. 2003. http://www.ema.europa.eu/docs/en_GB/document_library/Referrals_document/Roaccutane_30/WC500010806.pdf (12 December 2011, date last accessed).

38 Vahlquist A, Kuenzli S, Saurat J. Chapter 229. Retinoids. In: Wolff K, Goldsmith LA, Katz SI *et al*., eds. *Fitzpatrick's Dermatology in General Medicine*. 7th edn. New York: McGraw-Hill, 2008. http://www.accessmedicine.com/content.aspx?aID=2970300 (12 December 2011, date last accessed).

39 Wiegand UW, Chou RC. Pharmacokinetics of oral isotretinoin. *J Am Acad Dermatol* 1998; **39**: S8–12.

40 Heinz N. Teratogenicity of isotretinoin revisited: Species variation and the role of all-trans-retinoic acid. *J Am Acad Dematol* 2001; **45**: S183–7.

41 Rozman B. Clinical pharmacokinetics of leflunomide. *Clin Pharmacokinet* 2002; **41**: 421–30.

42 European Medicines Agency (EMA). *European Public Assessment Report (EPAR) for Arava (Leflunomide) - Product Information*. 2009. http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/000235/WC500026289.pdf (9 December 2011, date last accessed).

43 Brent RL. Teratogen update: reproductive risks of leflunomide (Arava); a pyrimidine synthesis inhibitor: counseling women taking leflunomide before or during pregnancy and men taking leflunomide who are contemplating fathering a child. *Teratology* 2001; **63**: 106–12.

44 Krishnan K. PBPK models in reproductive and developmental toxicology. In: Gupta RC, ed. *Reproductive and Developmental Toxicology*. London: Elsevier, 2011.

45 Fisher JW, Whittaker TA, Taylor DH *et al*. Physiologically based pharmacokinetic modeling of the pregnant rat: a multiroute exposure model for trichloroethylene and its metabolite, trichloroacetic acid. *Toxicol Appl Pharmacol* 1989; **99**: 395–414.

46 Corley RA, Mast TJ, Carney EW *et al*. Evaluation of physiologically based models of pregnancy and lactation for their application in children's health risk assessments. *Crit Rev Toxicol* 2003; **33**: 137–211.

47 Marschner N, Kötting J, Eibl H *et al*. Distribution of hexadecylphosphocholine and octadecyl-methyl-glycero-3-phosphocholine in rat tissues during steady-state treatment. *Cancer Chemother Pharmacol* 1992; **31**: 18–22.

48 European Medicines Agency (EMA) - Committee for Medicinal Products for Human Use (CHMP). *Note for Guidance on Toxicokinetics: A Guidance For Assessing Systemic Exposure in Toxicology Studies*. 2006. http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500002770.pdf (12 December 2011, date last accessed).

49 Datta V, Chaturvedi P. Congenital malformations in rural Maharashtra. *Indian Pediatr* 2000; **37**: 998–1001.

50 Swain S, Agrawal A, Bhatia BD. Congenital malformations at birth. *Indian Pediatr* 1994; **31**: 1187–91.

51 EURO-PERISTAT Project in collaboration with SCPE, EUROCAT & EURONEOSTAT. *European Perinatal Health Report*. 2008. http://www.europeristat.com/bm.doc/european-perinatal-health-report.pdf (16 January 2012, date last accessed).

52 European Surveillance of Congenital Anomalies (EUROCAT). *Special Report: A Review of Environmental Risk Factors for Congenital Anomalies*. 2004. http://www.eurocat-network.eu/content/Special-Report-Env-Risk-I-and-II.pdf (17 January 2012, date last accessed).

53 van Thiel PP, Leenstra T, Kager PA *et al*. Miltefosine treatment of Leishmania major infection: an observational study involving Dutch military personnel returning from northern Afghanistan. *Clin Infect Dis* 2010; **50**: 80–3.

54 Dorlo TP, Huitema BD, Beijnen JH *et al*. Optimal dosing of miltefosine in children and adults with visceral leishmaniasis. *Antimicrob Agents Chemother* 2012; in press.