Clinical pharmacology in leishmaniasis: treatment optimization of a neglected disease

Dorlo, T.P.C.

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

Thomas P. C. Dorlo
Manica Balasegaram
María Angeles Lima
Peter J. de Vries
Jos H. Beijnen
Alwin D. R. Huitema


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Abstract

Background: Use of miltefosine in the treatment of visceral leishmaniasis (VL) is hampered by its potential teratogenicity. Duration of adequate contraceptive cover in females of child-bearing potential after cessation of a potentially teratogenic drug 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 and weight of 25 (IQR 16—31) yrs and 38 (IQR 34—42) kg, respectively. 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 2 months for the shorter regimens.

Conclusion: 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.
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 the use of miltefosine in the clinic, certainly in rural areas, is its potential reproductive toxicity, which was demonstrated in animal studies but remains to be confirmed in human. Foeto- and embryotoxicity were demonstrated in both rabbits and rats, while teratogenicity was shown in rats only 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 dismiss its use during pregnancy and recommend in 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 literature and guidelines even a period of 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 up until 5 months after end of therapy (150 mg/day for 28 days) [8]. Since the teratogenic effect-level for miltefosine remains unknown in human, 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 length and kind 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 not favourable and causes concerns for adherence (e.g. barrier or oral contraception); while too short a period may increase the risk at congenital malformations. Therefore, more knowledge is urgently needed on the expected miltefosine levels in women of child-bearing potential from areas where VL is endemic, which can 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 based on conversion and translation of dosing data from preclinical
reproductive toxicity studies in animals and simulation of human pharmacokinetic data using a comprehensive anthropometric dataset of an historic cohort of Indian VL patients.

Material and 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 years and ≤45 years]. All individuals with either weight or height missing were removed from this anthropometric dataset, because both values are needed to estimate the fat-free body mass (FFM) of each individual.

FFM in kilograms was estimated from total body weight (WT) in kilograms and height (H) in meters as follows [13]:

\[ FFM = WHS_{\text{max}} \cdot H^2 \cdot \left( \frac{WT}{WHS_{50} \cdot H^2 \cdot WT} \right) \]  
(Eq. 1)

Where, for females, WHS_{\text{max}} (maximal Weight for Height Standard) is 37.99 kg/m² and WHS_{50} (half maximal Weight for Height Standard) is 35.98 kg/m² [13].

Simulations using a population pharmacokinetic model

All calculations, simulations and estimations were performed on a dual-core desktop computer running NONMEM VII (level 2.0) [14], the R statistical software package (version 2.14.0; http:// http://www.r-project.org/) [15], and Perl speaks NONMEM (PsN, version 2.3.1; http://psn.sourceforge.net) [16]. Pirana (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 output from NONMEM was processed, interpreted and visualized with R. Visual predictive checks were also made using R. An open two-compartment model with first-order absorption and elimination from the central compartment, which was estimated and validated from previous miltefosine pharmacokinetic data from Indian individuals [18], was used for the simulations. 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 sizes. E.g. for drug clearance the following equation was used:

\[
\frac{CL}{F_i} = \theta_1 \left( \frac{FFM_i}{FFM_{std}} \right)^{PWR} \cdot \exp(\eta_{i, CL/F})
\]  

(Eq. 2)

Where \( \frac{CL}{F_i} \) represents the clearance of the \( i \)th individual, \( \theta_1 \) is the typical value of clearance and \( \eta_{i, CL} \) is the between-subject random effect with a mean of 0 and a variance of \( \omega^2 \). \( FFM_i \) is the calculated fat-free body mass of the \( i \)th individual (see Eq. 1), \( FFM_{std} \) is a standard fat free body mass (arbitrarily 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 the biological principles that support these values [19–22]. The following estimates for the 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 the bioavailability were used (\( CL/F \), \( V/F \), etc.).

**Table 1.** Final population pharmacokinetic parameter estimates. Final estimates from the miltefosine population pharmacokinetic model with 2 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 and 1 for \( CL/F \) and \( V/F \), respectively [18].

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate</th>
<th>BSV (%)</th>
<th>Estimate uncertainty (RSE [%])</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absorption rate ( (k_a) ) (h(^{-1} ))</td>
<td>0.416</td>
<td>18.2</td>
<td>11.5</td>
</tr>
<tr>
<td>Clearance ( (CL/F) ) (liters/day)</td>
<td>3.99</td>
<td>32.1</td>
<td>3.5</td>
</tr>
<tr>
<td>Volume of central compartment ( (V_2/F) ) (liters)</td>
<td>40.1</td>
<td>34.1</td>
<td>4.5</td>
</tr>
<tr>
<td>Intercompartmental clearance ( (Q/F) ) (liters/day)</td>
<td>0.0347</td>
<td>NE</td>
<td>18.3</td>
</tr>
<tr>
<td>Volume of peripheral compartment ( (V_3/F) ) (liters)</td>
<td>1.75</td>
<td>NE</td>
<td>8.2</td>
</tr>
<tr>
<td>Residual variability (%)</td>
<td>34.3</td>
<td>NE</td>
<td>3.7</td>
</tr>
</tbody>
</table>

\(^{a}\) Between-subject variabilities in \( CL/F \) and \( V/F \) were correlated with a correlation coefficient of 0.92
NE, not estimated; BSV, between-subject variability; RSE, relative standard error
Simulated miltefosine dose regimens

Several lengths or 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, conform the current guidelines for miltefosine usage in India: individuals with a body weight below 25 kg were allocated to receive 50 mg miltefosine once daily; while individuals with a body weight ≥ 25 kg were allocated to receive 50 mg twice daily with a 12 hour interval, which is a total of 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, for which specifically no reproductive toxicity in the most sensitive animal test species (the rat) was observed, was determined from previous preclinical teratogenicity, foeto- and embryotoxicity studies. This NOAEL was translated to a total human equivalent dose using the dose calculator tool available through the FDA [23]. Basically, the total dose per body weight in rats (mg/kg) was recalculated to a total dose per body surface area (BSA) in rats (mg/m²) using a default rat body weight of 0.15 kg and BSA of 0.025 m² [24,25]. This dose per BSA was converted to a total NOAEL human equivalent dose using Boyd’s formula for BSA [26], as shown in Equation 3:

\[
HED = \frac{Dose_{rat} \cdot WT_{rat}}{BSA_{rat}} \cdot (0.0003207 \cdot W_{\text{human}}^{(0.7285 - 0.0188 \log_{10} WT_{\text{human}})} \cdot HT_{\text{human}}^{0.3}) 
\]  

(Eq. 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-∞_) 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 human 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 human and the animal test species, the reproductive safety threshold exposure limit was divided by a default animal-to-human uncertainty factor of 10 [27–31].
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 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$_{EOC-\infty}$’s were calculated in NONMEM by integrating the amounts in dummy compartments, according to Equation 4:

\[
AUC_{EOC-\infty} = \int_{EOC}^{\infty} C_i \cdot dt
\]

(Eq. 4)

Figure 1. Schematic depiction of contraceptive cover in relation to miltefosine exposure. The figure shows a typical miltefosine plasma concentration versus time curve. Contraceptive cover is required during treatment plus an extra (variable) period after the end of treatment (indicated in between the dashed lines). The miltefosine exposure (1) during treatment with contraceptive cover is indicated in white, (2) after end of treatment (EOT) but still with contraceptive cover in light grey and (3) after end of treatment and after end of contraception in dark grey. In this study we focused on the miltefosine area under the curve from the end of contraception (EOC) until infinity (AUC$_{EOC-\infty}$) for different durations of miltefosine treatment and different durations of contraceptive cover period.
The individual $\text{AUC}_{\text{EOC-}\infty}$ was compared to the reproductive safety threshold exposure limit. The probability for simulated Indian female VL patients of child-bearing potential of having an exposure exceeding the threshold exposure limit was calculated for all of the four different treatment durations and all three considered 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 ($\geq 12$ years and $\leq 45$ years). The most salient demographic characteristics of the selected female VL patients of child-bearing potential are depicted in Table 2.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Median value (interquartile range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>25 (16 – 31)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>38 (34 – 42)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>148 (144 – 152)</td>
</tr>
<tr>
<td>Body Mass Index [BMI] (kg/m$^2$)</td>
<td>17.3 (15.8 – 18.8)</td>
</tr>
<tr>
<td>Fat-free body mass (kg)</td>
<td>27.1 (24.6 – 29.5)</td>
</tr>
</tbody>
</table>

### Population pharmacokinetic Monte Carlo simulations

Of the 465 selected women, only 21 (4.5%) had a body weight lower than 25 kg and were allocated a miltefosine dosage of 50 mg once daily, 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 (range 2.08-2.63 mg/kg) for body weights below 25 kg and 2.67 mg/kg (range 1.42-4.00 mg/kg) for body weights equal or above 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). Visual predictive checks (Figure 2) extracted from these simulations ($n = 465 \times 100$) depict the median concentrations during and after treatment and the 90% prediction interval for the respective dose regimens. Simulated miltefosine plasma concentrations at
Figure 2. Monte Carlo simulations of miltefosine concentration-time curves for various dosing regimens. Median individual predicted miltefosine plasma concentrations (dark line) and 90% prediction intervals (grey area) after different miltefosine dosing regimens (5, 7, 10 and 28 days) in Indian female VL patients of childbearing potential (12 years ≤ age ≤ 45 years). The broken lines indicate the current lower limit of quantitation of miltefosine in plasma (4 ng/mL) [32].
various time points after start of treatment were evaluated and are shown in Table 3. Additionally, the time until the simulated miltefosine plasma concentration curves reached the lower limit of quantitation of the currently most sensitive detection method for miltefosine in human plasma (4 ng/mL) [32], i.e. miltefosine concentrations would become undetectable, was for the 5, 7, 10 and 28 day regimens after a median time (90% prediction interval) of 158 days (103-216 days), 176 days (119-235 days), 196 days (139-255 days) and 258 days (201-318 days), respectively.

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

In reproductive animal studies, oral doses of 1.2 mg/kg/day and above given for 10 days to pregnant rats minimally led to teratogenicity, the maximal miltefosine dose that caused no reproductive toxicity (NOAEL) was therefore 0.6 mg/kg/day [3,33,34]. Given the pharmacokinetic properties of miltefosine, with an extremely long primary and terminal elimination half-life and thus a high accumulation of subsequent dosages, a single total dose of miltefosine was regarded as equivalent in terms of total exposure to the same total dosage divided over e.g. 10 days. Therefore, in rat (body weight and BSA fixed at 0.15 kg and 0.025 m², respectively), 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/m² in rats. Converting this NOAEL BSA-normalized dose to a human equivalent dose (body weight and height fixed at 38 kg and 148 cm, respectively), a 36 mg/m² dose would result in a total single human equivalent dose of 45 mg, which corresponds to 50 mg if rounded to the nearest miltefosine capsule unit. The median AUCₐ₋ₐ (90% prediction interval) following administration of 50 mg in the selection of Indian female VL patients of child-bearing potential (n = 465) was 245 µg·day/mL (140 – 467 µg·day/mL). Divided by an animal-to-human uncertainty factor of 10, the reproductive safety threshold exposure limit was identified at 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 exposure after the end of different contraceptive cover durations until infinity (AUC_{EOC-∞}) was analyzed (Table 4 and Figure 1). The probability for the simulated Indian female VL patients of child-bearing potential of having a post-contraceptive (or ‘unprotected’) exposure to miltefosine higher than the identified reproductive safety threshold exposure limit.

Table 4. Exposure to miltefosine (area under the curve after end of contraception [EOC] until infinity [AUC_{EOC-∞}]) after different miltefosine regimens (5, 7, 10 or 28 days) simulated in typical Indian female VL patients of child-bearing potential.

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

AUC_{EOC-∞}, area under the curve after end of contraception until infinity; PI, prediction interval

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

<table>
<thead>
<tr>
<th>Miltefosine regimen</th>
<th>Probability of exposure above the reproductive safety threshold exposure limit No. of months on contraception after EOT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 month</td>
</tr>
<tr>
<td>5 days</td>
<td>4.3%</td>
</tr>
<tr>
<td>7 days</td>
<td>18.2%</td>
</tr>
<tr>
<td>10 days</td>
<td>54.6%</td>
</tr>
<tr>
<td>28 days</td>
<td>93.6%</td>
</tr>
</tbody>
</table>

Figures in bold type indicate probabilities exceeding the acceptable upper limit of probability for supra-threshold miltefosine exposure, which was set at 1/10th of the overall congenital malformation rate (i.e. 0.244%).

EOT, end of therapy
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 regimes. For the 5 and 7 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 <0.1 %, while for the 10 and 28 day regimen 3 and 4 months of contraceptive cover, respectively, were needed to reach <0.1% probability (Table 5).

Conclusion and 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 human. A reproductive safety threshold exposure limit was defined for miltefosine based on the conversion of the NOAEL dose in animal reproductive toxicity studies to human. Miltefosine exposure was simulated for Indian female VL patients of child-bearing potential following treatment with different miltefosine regimens 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 miltefosine regimen.

The design of teratogenic risk management-programmes and -strategies for drugs exhibiting reproductive toxicity in preclinical studies is problematic, often lacking rational considerations and usually not taking into account any actual data from preclinical studies. Human maternal or foetal pharmacokinetic data are very rarely available for those compounds, which could facilitate the estimation of a minimal human teratogenic effect level of the drug. Even animal pharmacokinetic data from reproductive toxicity studies are most often lacking or at least not made publicly available, complicating extrapolation of the teratogenic dose-effect relationship [35]. 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 [36,37]. For isotretinoin, a vitamin A derivative used in the treatment of cystic acne vulgaris, the time until (endogenous) retinoic acid levels have returned to normal after end of treatment is taken as directive to define the reproductive safety period [38–41]. Another related approach is based on the time needed until the drug becomes undetectable in plasma, which is used e.g. for leflunomide, a pyrimidine synthesis inhibitor in the treatment of rheumatoid
Several disadvantages are associated with this latter approach. Firstly, with inevitably increasing sensitivity of analytical techniques and equipment, the time period until the drug becomes undetectable will increase simultaneously and thus recommendations based on this approach are inclined to change over time. Secondly, the bioanalytical lower limit of quantitation in plasma is not necessarily related to any teratogenic concentration-effect relationship and does not exclude relative drug accumulation in the uterus or foetus, 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 e.g. foetal exposure and lactational transfer, but have rarely been used in the development of teratogenic risk management-strategies of drugs [45–47]. In the current study, the NOAEL dose in animal reproductive studies was used to determine a reproductive safety threshold exposure limit for miltefosine in human. 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 the drug elimination half-life does not allow 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 how long and which contraceptive cover methods are to be implemented in miltefosine treatment guidelines.

Nevertheless, these suggestions should also be interpreted with caution because of some important study assumptions and limitations. First, determination of the maximal safe miltefosine dose in pregnant female rats was previously performed in a small set of animals and should be regarded as presumptive evidence, which is actually a more general limitation of preclinical reproductive toxicity studies. On the other hand, dose administration in teratogenicity studies was performed for an extended critical period of time during gestation taking into account worst-case scenarios [3]. Additionally, a default well-supported animal-to-human uncertainty factor of 10 was applied [25,30,31]. Second, it must be considered that animal dose regimens remain difficult to extrapolate to human without any further data on miltefosine pharmacokinetics in either pregnant animals or human, although there is no current evidence that miltefosine distribution and metabolism, mainly through phospholipases, is significantly different in any other animal species compared to human [3,48]. Nevertheless, the probability analysis presented here could have been
improved significantly if more preclinical data on foetal or maternal drug levels of miltefosine in the reproductive toxicity studies in animals would have been available to incorporate into a PBPK model. This would have allowed the extrapolation of a concentration-effect relationship, in contrast to 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 this specific purpose in regulatory guidelines [49]. Third, the pharmacokinetic model that we employed in this study was previously estimated from data from European adult males, Indian adult males and Indian children [8,18]. Until now no data have been collected on miltefosine pharmacokinetics in females. Again based on the known pathways of metabolism of miltefosine, any differences between the population pharmacokinetic model parameter estimates of males and females are not expected. Evaluation of pharmacokinetics should be prioritized during drug development for neglected tropical diseases, specifically also 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 be taken into account as well, since this is defined by 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% [50,51]. In Europe, a more accurate overall incidence of 2.44% was reported [52], which may be explained by a higher autopsy rate in the included European centres. Around ~10% of congenital malformations are environmentally induced [53]. To define contraceptive adequacy, it might therefore be appropriate to set the acceptable upper limit of probability for supra-threshold miltefosine exposure at 1/10th of the overall congenital malformation rate, which is 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 will be the type of contraceptive cover. Tools 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 sterilisation may be too long for the period of cover required. Depot contraceptives (e.g. medroxyprogesterone acetate) may provide adequate coverage for 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 available at local endemic country level.

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 an absence of a 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 of ejaculate [54]. Although nothing is known
about sperm count and quality in these patients, this finding does clearly point at
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 e.g. animal studies and seminal DNA quality of male patients 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 minimal safe dose in reproductive toxicity studies in animals to a reproductive
safety threshold exposure limit in human. 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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