*J Antimicrob Chemother* 2016; **71**: 1892–1898
doi:10.1093/jac/dkw089 Advance Access publication 15 April 2016

Journal of Antimicrobial Chemotherapy

# Pharmacokinetics and pharmacodynamics of oleylphosphocholine in a hamster model of visceral leishmaniasis

**Anny Fortin[1,2]*, Thomas P. C. Dorlo[3,4], Sarah Hendrickx[5] and Louis Maes[5]**

[1]*Dafra Pharma Research & Development, Slachthuisstraat 30/7, Turnhout, Belgium;* [2]*McGill University, Department of Biochemistry, 1650 Cedar Ave, Montreal, Quebec, Canada;* [3]*Utrecht Institute for Pharmaceutical Sciences, Division of Pharmacoepidemiology & Clinical Pharmacology, Utrecht University, Utrecht, The Netherlands;* [4]*Department of Pharmaceutical Biosciences, Uppsala University, Uppsala, Sweden;* [5]*Laboratory for Microbiology, Parasitology and Hygiene (LMPH), University of Antwerp, Universiteitsplein 1, Wilrijk-Antwerp, Belgium*

*Corresponding author. Tel: +32-14-61-78-20; Fax: +32-14-47-29-90; E-mail: anny.fortin@mcgill.ca

*Received 13 July 2015; returned 23 October 2015; revised 16 February 2016; accepted 29 February 2016*

**Objectives:** This study evaluated the pharmacokinetic properties of oleylphosphocholine (OlPC) in hamsters following a single oral dose. Its prophylactic activity was tested to establish exposure–activity relationships, while a 5+5 day oral regimen at 20 mg/kg with long post-treatment follow-up was performed to assess its curative potential.

**Methods:** Single oral doses of 20, 50 and 100 mg/kg were administered for pharmacokinetic analysis while a 100 mg/kg single oral dose was given on day 7, 4 or 1, or 4 h prior to infection in the prophylactic efficacy study. The animals were infected on day 0 with *Leishmania infantum* and the resulting parasite burdens were measured in target organs on day 21. In the curative model, treatment started on day 21 post-infection at 20 mg/kg for 5+5 days and amastigote burdens were determined in target organs either on day 42 [10 days after the end of treatment (dpt)] or day 72 (40 dpt).

**Results:** OlPC showed elimination $t_{1/2}$ of ∼50 h and dose-proportional exposure. The prophylactic action of OlPC was in agreement with model-simulated drug exposures, showing dose-dependent residual activity. Interestingly, the 100 mg/kg single dose administered 4 days before infection (day −4) still reduced the overall parasite burden by ∼50%. In the curative model, >99% clearance of infection was observed at 10 dpt in all OlPC-treated animals and remained so at 40 dpt.

**Conclusions:** This study reveals that total plasma exposure ($AUC_{t-\infty}$) correlates well with the prophylactic and curative efficacy of OlPC in the *L. infantum* hamster model.

## Introduction

The neglected tropical disease leishmaniasis is prevalent in 98 countries and three territories spread over five continents and is most frequent among the most vulnerable poorer populations of East Africa, the Indian subcontinent and South America.[1,2] Worldwide, most recent counts indicated more than 58000 visceral leishmaniasis (VL) and 220000 cutaneous leishmaniasis cases per year.[1] VL is fatal if left untreated and is caused by the *Leishmania donovani* complex in East Africa and the Indian subcontinent and *L. infantum* in Europe, North Africa and Latin America.[3] The current treatment options for VL are the pentavalent antimonials, amphotericin B and its lipid formulations, paromomycin and the alkylphosphocholine, miltefosine. In general, these therapies present several problems for patient management in low-income countries including toxicity, adverse effects, high cost, long treatment regimens often requiring hospitalization, and development of drug resistance.[4]

Oleylphosphocholine (OlPC) is an alkylphosphocholine structurally related to miltefosine, but a distinct chemical entity.[5] Based on data accumulated from two independent rodent models of *L. infantum* visceral infection in Golden hamsters[5] and *L. major* cutaneous infection in BALB/c mice,[6] OlPC was shown to have better *in vivo* efficacy compared with miltefosine at equivalent doses. Although no direct comparison with miltefosine was performed, the clinical efficacy of OlPC against *L. infantum* canine leishmaniasis was demonstrated in naturally infected dogs using a 14 day regimen at 4 mg/kg/day,[7] a daily dose exceeding the maximum tolerated dose of miltefosine in that species (recommended regimen in dogs: 2 mg/kg for 28 days). Cidal activity was demonstrated against promastigotes and intracellular amastigotes and shown to be independent from host factors.[5,8]

Because of its amphiphilic nature, OlPC can be diluted in water for dosing or be formulated in liposomes in which the drug molecules become part of a double-layer membrane. The OlPC

liposomes can be administered not only orally but also intravenously without causing haemolysis, a problem currently associated with the alkylphosphocholines.[9] In previous preclinical experiments, both aqueous and liposomal formulations of OlPC given orally were shown to be efficacious and safe in the hamster model of VL, with a slight advantage for the liposomal formulation.[5]

Comparative safety and efficacy data collected so far in animal models suggest that OlPC has a wider therapeutic window compared with miltefosine.[5–7] Since the antileishmanial activity of OlPC and miltefosine are comparable *in vitro* against intracellular amastigotes, the observed difference in therapeutic index could logically result from differences in pharmacokinetics (PK) (e.g. oral bioavailability, tissue distribution) or its pharmacodynamics (PD) at the level of host cells. Studying the PK/PD of OlPC in its different formulations will allow a better understanding of the disposition and action of the drug and will be helpful in translating the knowledge accumulated in animals to future clinical studies in patients. As a first step towards this translational goal, the current study reports PK properties of OlPC in hamsters following single oral dose administration at various dose levels of two different formulations. Subsequently, a population PK model was developed to allow simulations of multiple dosing regimens. Given the long half-life of OlPC (50 h), a prophylactic dosing scheme was designed to explore the relationship between drug exposure and overall residual efficacy in hamsters treated at different timepoints before infection. Finally, the curative efficacy following multiple administrations was evaluated, including long post-treatment follow-up and matched to the model-simulated exposure to OlPC.

## Materials and methods

### Animals

Female Golden hamsters were purchased from Janvier (Le Genest, France) and kept in quarantine for at least 1 week before starting the experiments. Food for laboratory rodents (Carfil, Arendonk, Belgium) and drinking water was available *ad libitum*. Animal experiments were carried out in strict accordance to all mandatory guidelines [EU directives, including the Revised Directive 2010/63/EU on the Protection of Animals used for Scientific Purposes that came into force on 1 January 2013, and the Declaration of Helsinki in its latest version) and were approved by the ethics committee of the University of Antwerp, Belgium (UA-ECD 2010–17, 18 August 2010)]. The animals were cared for in line with national guidance. The authors abide to the reductionist approach of using animal models in drug development.

### Drug products

Miltefosine (Sigma–Aldrich) and OlPC (Dafra Pharma International) stock solutions were prepared in water and stored at 4–8°C in the dark for a maximum of 7 days. The OlPC liposomes were also provided by Dafra Pharma International. Briefly, the liposomes consisted of OlPC, cholesterol (Solvay Pharmaceuticals, Switzerland) and oleic acid (Hana Corporation, Korea) in a molar ratio of 41.51:42.42:5.13. They were produced by a cross-flow injection technique (Polymun Scientific, Austria) at a concentration of 18 mg/mL. OlPC liposomes showed a mean diameter 75 nm as measured by dynamic light-scattering analysis with a Malvern Nano ZS particle sizer (Malvern Instruments Ltd, UK). The formulation was sterile-filtrated and stored at 4–8°C until use. If needed for experimentations, it was further diluted in PBS and immediately used.

### Single-dose PK studies

Hamsters were randomly allocated to experimental groups (four to eight per group) based on their body weight. At the start of the experiment, each animal received a single dose of OlPC in the aqueous or liposomal forms by oral gavage. Dosages were adapted to individual weight. Intracardiac punctures for PK sampling were performed by a skilled technician under transient isoflurane anaesthesia and using an aseptic technique, and maximal care was taken to minimize animal suffering. Blood samples (150 μL) were collected in EDTA-coated 1.3 mL microtubes (Sarstedt). After centrifugation for 10 min, the plasma samples were further processed for UPLC analysis, or stored at −20°C until analysis. A maximum of 6 samples, taken at 2, 6, 24, 32, 72 and 168 h post-dosing, were evaluated for each hamster. Single doses of 20, 50 and 100 mg/kg were tested in three independent replicate experiments.

### Bioanalysis

Protein precipitation was used as standard processing technique: four parts of cold acetonitrile added to one part of plasma. The mixture was then vortexed for 30 s and centrifuged at 4°C for 5 min. The supernatant was stored at −20°C until analysis. The bio-analysis was done by liquid chromatography (UPLC) (Waters AquityTM) coupled with tandem quadrupole mass spectrometry ($MS^2$) (Waters XevoTM), equipped with an electrospray ionization interface and operated in multiple reaction monitoring mode. For control of the chromatographic separation conditions, a generic method was used with the following solvents: solvent A = water + 0.05% (v/v) ammonium hydroxide ($NH_4OH$) and solvent B = methanol + 0.05% (v/v) $NH_4OH$. The initial flow was set at 0.6 mL/min 30% A + 70% B. The linear gradient was 1.3 min to 5% A + 95% B; 1.7 min at 0% A + 100% B and 1 min equilibration at 30% A + 70% B. For calibration, standard curves in blank hamster plasma were made in duplicate and covered concentration ranges between 0.625 and 62 500 ng/mL. The calibration curve was linear over the range 0.5–100 nM with a lower limit of quantification of 0.5 nM. The standard calibrator concentrations were within 20% of the nominal concentration at a lower limit of quantification and within 15% of the nominal concentration at all other concentrations. No internal standard was used.

### PK analysis

Population PK of OlPC was performed with non-linear mixed effects modelling using the first-order conditional estimation method with

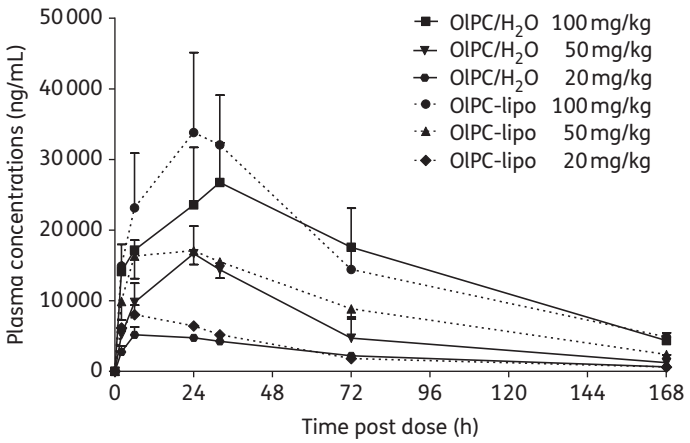


**Figure 1.** Concentration–time curves of observed plasma concentrations in hamsters following single oral administration of 20, 50 or 100 mg/kg of two formulations of OlPC. OlPC in an aqueous solution ($OlPC/H_2O$) curves are presented as solid lines while OlPC in a liposomal formulation (OlPC-lipo) curves are presented as dashed lines ($n = 2–4$/group).

**Table 1.** Standard pharmacokinetic parameters following single dose administration of oleylphosphocholine (OlPC) in female hamsters at 20, 50 or 100 mg/kg

| | 20 mg/kg | | 50 mg/kg | | 100 mg/kg | |
|---|---|---|---|---|---|---|
| Parameter | OlPC/$H_2O$ ($n=4$) | OlPC-lipo ($n=2$) | OlPC/$H_2O$ ($n=3$) | OlPC-lipo ($n=3$) | OlPC/$H_2O$ ($n=2$) | OlPC-lipo ($n=4$) |
| Weight, g | 83.8 ± 4.6 | 82 ± 5.7 | 74 ± 1.7 | 76 ± 3.0 | 72 ± 2.8 | 72.8 ± 1.5 |
| $C_{max}$, ng/mL | 5475 ± 717 | 8045 ± 1336 | 17063 ± 3577 | 17787 ± 1487 | 26755 ± 5621 | 38348 ± 5349 |
| $T_{max}$, h | 10.5 ± 9 | 6 ± 0 | 26.6 ± 4.6 | 14.6 ± 15 | 31.9 ± 0 | 26 ± 4 |
| $AUC_{\infty}$, ng·h/mL | 474522 ± 22629 | 583392 ± 6788 | 1199384 ± 55515 | 1561912 ± 122901 | 2410104 ± 142858 | 3049860 ± 188353 |
| $t_{1/2}$, h | 47.4 ± 0.7 | 44.27 ± 1.2 | 51.2 ± 1.8 | 48.6 ± 2.0 | 51.8 ± 1.3 | 50.1 ± 1.9 |

$C_{max}$ and $T_{max}$ are derived from observed values while AUC and $t_{1/2}$ are derived from individual model parameter estimations as described in the Materials and methods section.
Values shown are mean ± SD.

interaction between random effects and residual variability as implemented in NONMEM (version 7.3, ICON Development Solutions, USA). Dataset management and graphical analysis was performed in R (version 3.1.2). The modelling process was further automated by the use of Perl-speaks-NONMEM (version 4.3.7), Pirana (version 2.9.0) and the R-package Xpose (version 4.5.3). Model development was initially guided by physiological and biological plausibility, the objective function value (OFV) as computed by NONMEM, corresponding to minus twice the log-likelihood (a ΔOFV of −3.84 with 1 degree of freedom, $\chi^2$ distribution, corresponds with a significance level of $P=0.05$) and standard basic goodness-of-fit plots. The predictive properties of the final model were evaluated using a visual predictive check (VPC) assessment based on 1000 simulations using the final model. In this VPC observed data were compared to the model simulated data to evaluate the predictive performance of the final model. The final population PK model estimates were used to perform simulations of the multiple-dose administrations in hamsters.

### *Single-dose prophylactic administration model*

Hamsters were randomly allocated to five groups (six to seven per group) and received a single dose of 100 mg/kg by oral gavage on day 7, 4 or 1, or 4 h prior to infection. One group was treated with water (vehicle-treated infected control or VIC). On day 0, the animals were infected intracardially (under fluothane anaesthesia) with $2\times10^7$ amastigotes of *L. infantum* obtained from the spleen of a heavily infected donor hamster. The animals were observed daily for the occurrence of clinical or adverse effects and weighed twice weekly during the further course of the experiment. On day 21, the animals were humanely euthanized by $CO_2$ overdose and the liver, spleen and bone marrow were collected. Impression smears were fixed in methanol and stained with Giemsa for microscopic enumeration of the number of amastigotes per cell by counting a minimum of 500 nuclei. The results from the spleen and liver are expressed as Leishman Donovan units (LDU) = mean number of amastigotes per nucleus × mg organ weight while those of the bone marrow are expressed in amastigotes per nucleus. The percentage reduction as compared with VIC was used as a measure for the drug efficacy.[10]

### *Multiple-dose administration in a long-term follow-up curative model*

Animals were randomly allocated to eight groups (six per group) and infected as described above. Treatment started on day 21 post-infection by oral gavage and the animals were monitored for adverse or clinical effects until the end of the experiment. Aqueous (OlPC/$H_2O$) and liposomal OlPC and aqueous miltefosine were dosed at 20 mg/kg for 5 + 5 days (i.e. days 21–25 and 28–32 post-infection). This regimen was specifically selected because it represents the same total dose and exposure as 40 mg/kg for 5 consecutive days (the latter being the standard scheme for miltefosine in the hamster model) but involves a lower $C_{max}$. All animals were weighed twice weekly to monitor general health status. Amastigote burdens in the liver, spleen and bone marrow were evaluated on day 42 (10 days after the end of treatment, dpt) or day 72 (40 dpt).

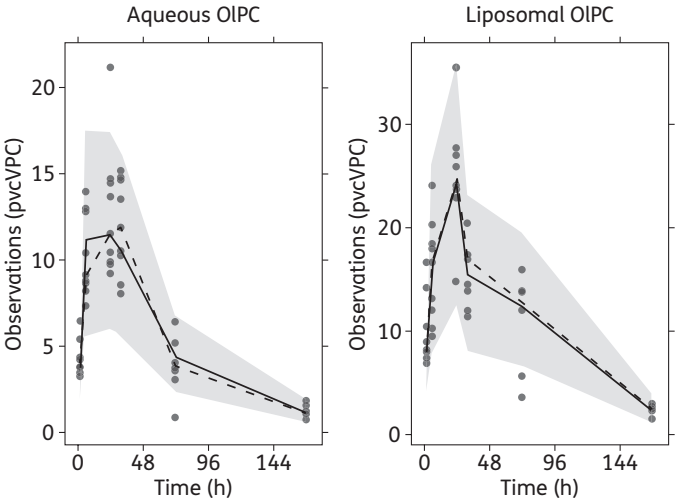


**Figure 2.** Assessment of the validity of the population PK model for the two OlPC formulations. Visual predictive checks (VPCs) based on 1000 Monte Carlo simulations were used to assess the validity of the population PK model. Prediction- and variability correction (pvcVPC) were applied to compensate for multiple-dose levels and the dataset was binned according to the time after dose administration. Grey area indicates the 95% prediction interval based on the simulations. Dashed line indicates the observed (real) median and the solid line indicates the simulated median.

## Results

### *PK characteristics of OlPC in hamsters*

The standard PK parameters associated with single oral doses of 20, 50 and 100 mg/kg OlPC were determined in hamsters using

aqueous (OlPC/$H_2O$) and liposomal formulations, which were used in parallel in three replicate experiments. Figure 1 presents the concentration–time curves of the three doses with the calculated PK parameters presented in Table 1. For the doses/formulations tested, $T_{max}$ varied between 10 and 32 h for OlPC/$H_2O$ and 6–26 h for OlPC liposomes, and half-lives were similar at roughly 50 h. Based on $C_{max}$ and $AUC_{0-\infty}$, dose proportionality was verified between 20, 50 and 100 mg/kg for both formulations. There is a consistent trend for the liposomes to be more orally available than the aqueous form (Figure 1).

In a second step, the results obtained for each dose were analysed in a population PK approach to obtain population model estimates of the absorption, distribution, metabolism and excretion-related PK parameters. A one-compartment disposition model with first-order absorption and elimination best fitted the data. Final validity of the model is shown in Figure 2 with a VPC based on 1000 Monte Carlo simulations. The observed differences between the formulations were investigated as covariates on the estimated PK parameters, and a significant proportional effect of the formulation on relative bioavailability (*F*) could be identified, where the typical *F* for the liposomal formulation was 1.35 times [20.6% relative standard error (RSE); ΔOFV −14.0, 1 degree of freedom extra, $P<0.001$] higher than for the aqueous formulation, for which *F* was fixed to 1 (since the absolute bioavailability of OlPC in hamsters was unknown). The fact that all observations for both formulations were within the 95% prediction interval except for three and that the medians were overlapping showed that the model described the observed data adequately with no apparent systematic bias. The parameter estimates from the final model are presented in Table 2. Based on this analysis, the absorption rate ($k_a$) of OlPC in hamsters was 0.246 $h^{-1}$ and the apparent total body clearance (CL/*F*) 0.04291 L/h/kg. The central volume of distribution (V/*F*) was 3.026 L/kg, indicating that the drug is highly distributed.

**Table 2.** Population pharmacokinetic parameter estimates for OlPC in hamsters

| Parameter | Estimated value (%RSE) | Between-subject variability, %CV (%RSE) |
|---|---|---|
| CL/*F* (L/h/kg) | 0.04291 (5.6) | 10.1 (41.3) |
| *V*/*F* (L/kg) | 3.026 (6) | 5.5 (88.5) |
| $k_a$ ($h^{-1}$) | 0.246 (13.7) | 29.5 (30.2) |
| Proportional residual variability | 24.1% (12.29) | — |
| *F* (for aqueous formulation) | 1 (fixed) | — |
| Proportional difference in *F* for the liposomal formulation | 1.353 (20.6) | — |

CL/*F*, apparent total body clearance; %CV, percentage coefficient of variation; *F*, bioavailability; $k_a$, absorption rate; %RSE, percentage relative standard error; *V*/*F*, apparent central volume of distribution. CL/*F* and *V*/*F* were normalized to kg using a linear extrapolation from the typical hamster weight of 77 g.
Between-subject variability on CL/*F* and *V*/*F* was 82.8% correlated and on *V*/*F* and $k_a$ was 56.1% correlated.

## *Prophylactic administration of OlPC in hamsters*

Given the long half-life of OlPC, a prophylactic dosing scheme was designed to evaluate its residual activity after a single 100 mg/kg oral dose administered on day 7, 4 or 1, or 4 h prior to infection (Table 3). Animals treated 7 days prior to infection retained negligible drug activity (0%, 40% and 10% reduction in the liver, spleen and bone marrow), which is in agreement with the PK curve showing a low plasma concentration and residual drug exposure ($AUC_{168-\infty}$) of 228 334 ng·h/mL. On the other hand, the response to drug treatment at days 4 and 1, and 4 h prior to infection dose-dependently increased the prophylactic efficacy, reaching 92%, 99% and 94% of amastigote reduction in the liver, spleen and bone marrow when OlPC was given 4 h prior to infection. Interestingly, a 100 mg/kg dose administered 4 days prior to infection still reduced the overall parasite burden by roughly 50%. The correlation between residual drug activity and the corresponding simulated $AUC_{t-\infty}$ (extracted from Table 3) is plotted in Figure 3. Taking into account a cut-off >95% parasite reduction ($ED_{95}$) in the target organs, liver and spleen, as the limit for drug efficacy,[11] non-linear regression curves suggested that a total exposure (AUC) to OlPC >2 200 000 ng·h/mL [$\sim 10^{\log(AUC)=6.37}$] would be the minimum exposure target required to clear the parasitaemia in the hamster model considering initial *L. infantum* inoculum of $2\times10^7$ amastigotes.

**Table 3.** Residual exposure and activity of single dose of 100 mg/kg oral aqueous OlPC administered on day 7, 4 or 1, or 4 h prior to infection

| | Amastigote burden in target organs (mean ± SEM) and associated percentage reduction compared with vehicle-treated control | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Groups | liver | (%) | spleen | (%) | BM | (%) | $AUC_{t-\infty}$ (ng·h/mL)[a] | Time above $2\times IC_{50}$ (h) |
| VIC | 11 535 ± 2072 | — | 270 ± 82 | — | 1.3 ± 0.3 | — | — | — |
| OlPC/−7 days p.i. | 13 334 ± 1027 | 0 | 161 ± 39 | 40 | 1.2 ± 0.2 | 10 | 228 334 | 0 |
| OlPC/−4 days p.i. | 8975 ± 2048 | 22 | 66 ± 22 | 77 | 0.6 ± 0.2 | 57 | 633 864 | 64 |
| OlPC/−1 days p.i. | 5194 ± 1233 | 55 | 48 ± 28 | 82 | 0.4 ± 0.2 | 67 | 1 759 248 | 136 |
| OlPC/−4 h p.i. | 975 ± 421 | 92 | 2 ± 1 | 99 | 0.1 ± 0.1 | 94 | 2 283 288 | 156 |

Total AUCs were calculated for time of infection (*t*) to infinity (∞). Time after infection that OlPC concentration remains above twice the *in vitro* $IC_{50}$ (2× 1800 ng/mL)[5] is provided in the last column.
VIC, vehicle-treated infected control; BM, bone marrow; p.i., post-infection; OlPC, oleylphosphocholine.
[a]Simulated based on parameters of Table 2.

### *Multiple-dose administration in the curative model of infection*

Based on the population PK parameter estimates in hamsters, simulations were performed to determine drug exposure after multiple-dose regimens (Figure 4; Table 4) and showed that, for instance, 5×20 mg/kg OlPC/$H_2O$ and OlPC liposomes would result in a typical total $AUC_{0-\infty}$ of 2 330 400 ng·h/mL and 3 153 120 ng·h/mL, corresponding to respective times with plasma levels >2× $IC_{50}$ of 213 and 235 h. On the other hand, dosing OlPC/$H_2O$ at 5×40 mg/kg would provide an $AUC_{0-\infty}$ of 4 660 800 ng·h/mL and plasma concentrations >2× $IC_{50}$ for 264 h. The $AUC_{0-\infty}$ for OlPC liposomes would be 6 306 000 ng·h/mL with plasma concentrations >2× $IC_{50}$ for 286 h.

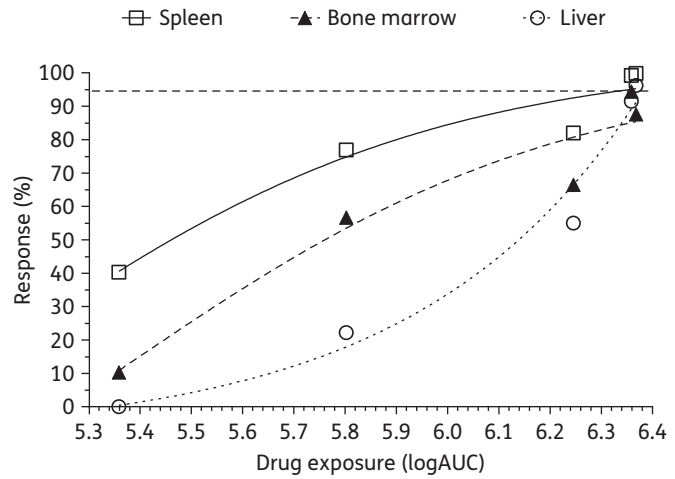


**Figure 3.** Relationship between total drug exposure ($AUC_{t-\infty}$) after infection and resulting parasitic reduction in animals treated prophylactically with OlPC. Liver, spleen and bone marrow of hamsters were harvested following a single prophylactic treatment of 100 mg/kg aqueous OlPC on day 7, 4 or 1, or 4 h prior to infection and parasitic loads at day 21 were compared with those of untreated control animals (*y*-axis). Corresponding residual exposure AUCs were extracted from data on non-infected hamsters, calculated for time of infection (on day 7, 4 or 1, or 4 h) to infinity (∞) (*x*-axis). Dashed line marks the typical cut-off >95% parasitic reduction in spleen and liver considered as limit for drug efficacy.

Since 5 days×40 mg/kg (total dose 200 mg/kg) has been previously associated with a complete clearance of all parasites in the hamster VL model,[5] this total dose was used to further explore the relationship between total exposure and overall efficacy. In a new curative experiment, the single regimen of 5+5 days×20 mg/kg of both OlPC formulations was tested (total dose 200 mg/kg, predicted $AUC_{0-\infty}$ 4 660 800 ng·h/mL and time >2× $IC_{50}$=388 h for OlPC/$H_2O$, and 6 306 000 ng·h/mL and time >2× $IC_{50}$=411 h for OlPC liposomes; Figure 4, Table 4). This time, the parasite burdens in target organs were evaluated for both 10 and 40 days after the end of treatment (dpt) to look at potential infection relapse. A complete clearance of the infection (100% reduction of Leishman Donovan units in all target organs) was observed with the 5+5 days×20 mg/kg regimens at 10 dpt in the OlPC liposome-treated group, and ≥99% reduction with OlPC/$H_2O$ group (Table 5). The parasite suppression remained the same when measured at 40 days post-treatment. These results are in agreement with those previously obtained with the 5 days×40 mg/kg regimen and are supported by the total exposure and total dose being the same in both experiments. Comparatively, miltefosine at 5+5 days×20 mg/kg demonstrated excellent efficacy at 10 dpt (98%, >99% and 90% reduction in the liver, spleen and bone marrow, respectively), but the parasitic loads increased again at 40 dpt (67%, 99% and 79%, respectively), reflecting relapse of the infection and hence inferiority to OlPC (Table 5).

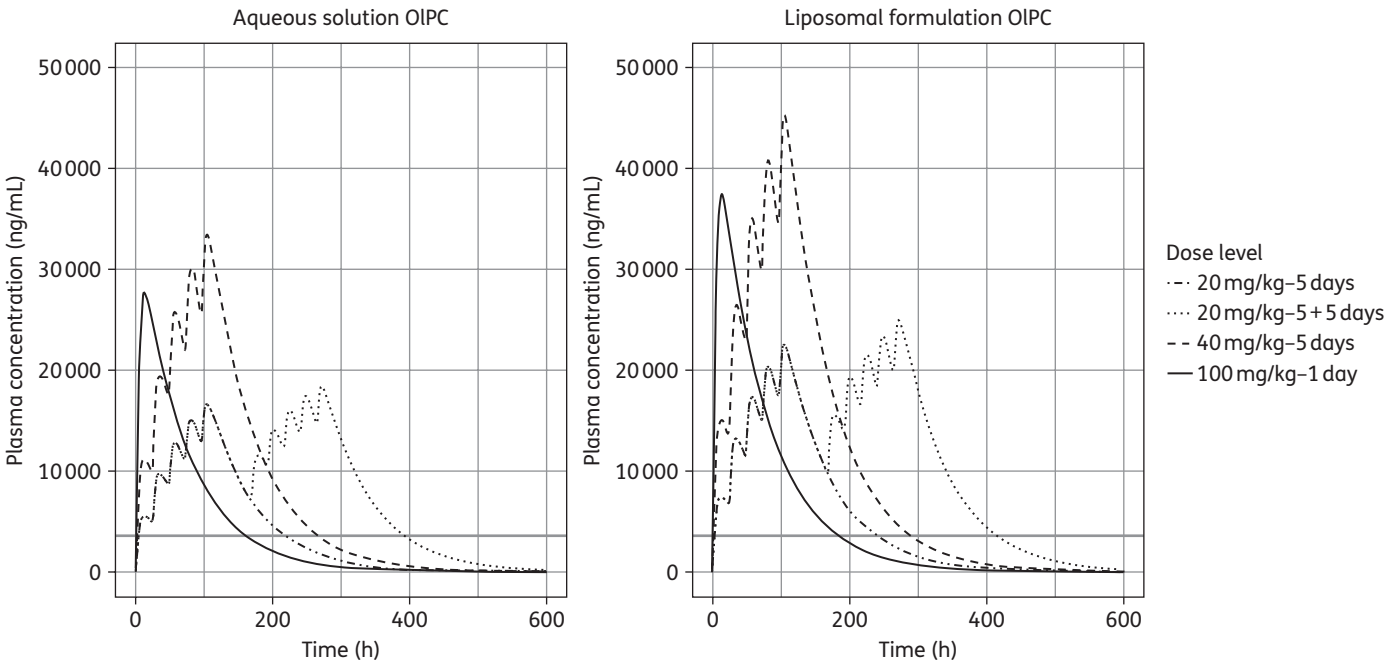


**Figure 4.** Simulated concentration–time plots of OlPC concentrations in hamsters following multiple oral administrations at various dose levels. Horizontal grey line represents the value corresponding with twice the *in vitro* $IC_{50}$ (2×1800 ng/mL).[5]

## Discussion

To our knowledge, this study describes for the first time the PK properties of oral OlPC in hamsters, the standard animal model for VL, and identified exposure–activity relationships for OlPC in *L. infantum*-infected hamsters. OlPC is highly distributed and has a relatively long plasma half-life of ∼50 h, meaning that a steady-state concentration will typically be achieved following 10 days of daily administration (i.e. 5× the elimination half-life for a one-compartment system). The fact that the PK parameters identified in non-infected hamsters were used to extrapolate the total exposure to OlPC in infected animals may represent a potential limitation of this study. Even though nothing has been documented for other drugs, it cannot be totally excluded that the presence of the infection might have some impact on OlPC metabolism (protein binding, absorption, etc.). On the other hand, obtaining PK data from infected animals is challenging, not only because the added stress of blood sampling and handling could impact the course of infection, but also because the state of infection itself is a dynamic event, which is highly variable in duration (model dependent), severity and outcome (species and strain dependent). We recognize that using cardiac puncture for PK sampling and parasite infection is a less appropriate and probably out-of-date practice from a welfare perspective, even when performed under the best possible conditions. In this regards, future studies will make use of an alternative blood sampling technique and route of infection, which are currently being implemented in our laboratory. Looking at the broader scope of the study, the use of PK modelling and simulation techniques did reduce suffering of animals, as extra blood taking for PK measurements was not needed.

Regarding the oral absorption mechanism, studies performed on the intestinal transport of miltefosine in Caco-2 cells suggest that miltefosine is absorbed both passively via the paracellular route (rapidly) and actively (more slowly) through a carrier-mediated pathway.[12,13] It is plausible that OlPC is absorbed by similar pathways. Since the PK of miltefosine in hamsters have not yet been published, it was not possible to compare to OlPC. Limited pre-clinical PK data of miltefosine have been summarized by Sindermann and Engel[14] who report a $T_{max}$ and plasma half-life of 4 and 84 h, and 48 and 159 h in rats and dogs, respectively.

In our PK study, the relative oral bioavailability of liposomal OlPC is shown to be ∼35% higher than the aqueous formulation, which may explain why the cure rates achieved using the liposomal formulation are higher than for the aqueous formulation at equivalent doses.[5] The exact cause of this increased bioavailability of the liposomes is unknown but could mechanistically be related to a more efficient passive absorption and/or enhanced active uptake of OlPC in liposomes compared with solution. This suggestion is supported by the fact that the $T_{max}$ seems earlier after liposomal administration compared with OlPC/$H_2O$ (Table 1). The

**Table 4.** Simulated drug exposures (AUC) for multiple-dose administrations of aqueous and liposomal oleylphosphocholine (OlPC) for a hamster (body weight 77 g)

| Dose level (mg/kg) | OlPC formulation | Regimen (days) | $AUC_{0-\infty}$ (ng·h/mL) | Time >2× $IC_{50}$ (h)[b] |
|---|---|---|---|---|
| 20 | $H_2O$ | 5 | 2330400 | 213 |
| 20 | $H_2O$ | 5+5 | 4660800 | 388 |
| 20 | Lipo | 5 | 3153120 | 235 |
| 20 | Lipo | 5+5 | 6306000 | 411 |
| 40 | $H_2O$ | 5 | 4660800 | 264 |
| 40 | Lipo | 5 | 6306000 | 286 |
| 100 | $H_2O$ | 1 | 2330400 | 160 |
| 100 | Lipo | 1 | 3153120 | 182 |

Simulations were based on the estimated typical population pharmacokinetic parameters as summarized in Table 2. Multiple-dose administrations were simulated for 5 days (1 dose/day) and for 5+5 days with 2 drug-free days in between (1 dose/day). Total AUCs were calculated for 0 to infinity (∞). Time that OlPC concentration remains above twice the *in vitro* $IC_{50}$ (2×1800 ng/mL)[a] is provided in the last column.

[a]From Fortin *et al*. 2012.[5]

[b]Calculated based on final typical population PK model predictions as the time OlPC concentrations were above 3600 ng/mL.

**Table 5.** Reduction of amastigote burdens in the liver, spleen and bone marrow on 10 and 40 days after end of treatment (dpt) following an oral dosing regimen of 20 mg/kg×5+5 days (i.e. day 21–25 and day 28–32) using aqueous (OlPC/$H_2O$) and liposomal (lipo) OlPC and miltefosine ($n$=5–6 animals per group)

| | Amastigote burdens in target organs (mean±SEM) and associated percentage reduction compared with vehicle-treated control | | | | | |
|---|---|---|---|---|---|---|
| Treatment group | liver | reduction (%) | spleen | reduction (%) | BM | reduction (%) |
| VIC −10 dpt | 3618±419 | — | 691±136 | — | 0.58±0.07 | — |
| VIC −40 dpt | 5566±1167 | — | 5712±1294 | — | 0.52±0.07 | — |
| MIL/$H_2O$ −10 dpt | 65±25 | 98 | 3.5±2 | >99 | 0.06±0.02 | 90 |
| MIL/$H_2O$ −40 dpt | 1856±1181 | 67 | 75±32 | 99 | 0.11±0.07 | 79 |
| OlPC/$H_2O$ −10 dpt | 0±0 | 100 | 2±1 | >99 | <0.01 | >99 |
| OlPC/$H_2O$ −40 dpt | 0±0 | 100 | 0.2±0.2 | >99 | 0.01±0.00 | 99 |
| OlPC-lipo −10 dpt | 0±0 | 100 | 0±0 | 100 | 0±0 | 100 |
| OlPC-lipo −40 dpt | 0±0 | 100 | 0±0 | 100 | 0±0 | 100 |

BM, bone marrow; VIC, vehicle-treated infected control; MIL, miltefosine; OlPC, oleylphosphocholine.

cholesterol component of the liposomal formulation could potentially play a role, but the observed effect is not in line with some *in vitro* results of Ménez *et al*.,[13] who reported that co-administration of miltefosine with cholesterol led to a large decrease of miltefosine incorporation in Caco-2 cells. Increased pre-systemic clearance of OlPC when administered in an aqueous solution is not considered plausible due to the extreme stability of the compound both *in vitro* as well as in *in vivo*.

The prophylactic efficacy scheme designed in the context of this project was not intended to assess the preventive capacity of the drug *per se* but rather to expose parasites to residual drug levels and examine resulting effects on parasite loads. In fact, the high dose of *ex vivo* amastigotes used for the infection actually does not reflect the natural situation where hosts are infected by the bite of a sandfly injecting metacyclic promastigotes. Despite the fact that our prophylactic model is much more stringent than would happen in the case of a natural infection, it still showed that a single dose of 100 mg/kg OlPC was able to reduce the overall parasite load by 50% in all target organs, even when given 4 days before infection. To date, no preventive treatment exists against leishmaniasis and may even be contraindicated for people living in disease-endemic areas because of the risk of selection for drug resistance. As for the theoretical consideration of a prophylactic treatment for travellers with an increased risk of exposure (military troops and humanitarian workers deployed in disease-endemic areas for limited periods), this would necessitate a favourable risk/benefit ratio and probably the presence of a companion drug to insure protection against resistance. In any case, potential drug candidate(s) would require to be tested in appropriate well-controlled models, which are currently not available.

Regarding the curative model adopting multiple administrations, the fact that regimens of 5 days×40 mg/kg[5] and 5+5 days×20 mg/kg of OlPC result in similar overall efficacy support the proposal that total exposure (AUC) is more important than $C_{max}$ for clearance of *L. infantum* infection. This is in agreement with what has been previously reported on the comparison of single and multiple administrations.[5] Since 20 mg/kg in hamsters corresponds to a human equivalent dose of 162 mg in a 60 kg adult,[15] and given the fact that miltefosine is used safely at daily dose of 150 mg, it is reasonable to accept that 100 mg could be used as a starting dose in human clinical trials aiming to study the safety and efficacy of OlPC in patients. However, in view of the known teratogenic potential of miltefosine,[16] the assessment of the teratogenic potential of OlPC is a pivotal point to consider in the progression of the clinical development of OlPC against VL.

## Acknowledgements

The authors are indebted to An Matheeussen, Pim-Bart Feijens and Mandy Vermont for expert technical assistance.

## Funding

This study was financed by grant no. 110402 from IWT, Flanders, Belgium (Innovatie door Wetenschap en Technologie) to A. F. at Dafra Pharma Research & Development (Turnhout, Belgium), by grant no. G051812N from FWO (Fonds Wetenschappelijk Onderzoek), Flanders, Belgium to L. M. at University of Antwerp (Antwerp, Belgium) and by Dafra Pharma International.

## Transparency declarations

A. F. works as a consultant for Dafra Pharma Research and Development. T. P. C. D. was a Scientific Advisory Board member for Dafra Pharma Research and Development. All other authors: none to declare.

## References

**1** Alvar J, Vélez ID, Bern C *et al*. Leishmaniasis worldwide and global estimates of its incidence. *PLoS One* 2012; **7**: e35671.

**2** Guerin PJ, Olliaro P, Sundar S *et al*. Visceral leishmaniasis: current status of control, diagnosis, and treatment, and a proposed research and development agenda. *Lancet Infect Dis* 2002; **2**: 494–501.

**3** WHO. Control of the leishmaniases. Report of a meeting of the WHO Expert Committee on the Control of Leishmaniases, Geneva, 22–26 March 2010. WHO Technical Report Series 949. http://apps.who.int/iris/bitstream/10665/44412/1/WHO_TRS_949_eng.pdf.

**4** van Griensven J, Balasegaram M, Meheus F *et al*. Combination therapy for visceral leishmaniasis. *Lancet Infect Dis* 2010; **10**: 184–94.

**5** Fortin A, Hendrickx S, Yardley V *et al*. Efficacy and tolerability of oleylphosphocholine (OlPC) in a laboratory model of visceral leishmaniasis. *J Antimicrob Chemother* 2012; **67**: 2707–12.

**6** Fortin A, Caridha DP, Leed S *et al*. Direct comparison of the efficacy and safety of oral treatments with oleylphosphocholine (OlPC) and miltefosine in a mouse model of *L. major* cutaneous leishmaniasis. *PLoS Negl Trop Dis* 2014; **8**: e3144.

**7** Hernández L, Gálvez R, Montoya A *et al*. First study on efficacy and tolerability of a new alkylphosphocholine molecule (oleylphosphocholine—OlPC) in the treatment of canine leishmaniosis due to *Leishmania infantum*. *Parasitol Res* 2014; **113**: 157–64.

**8** Shio MT, Paquet M, Martel C *et al*. Drug delivery by tattooing to treat cutaneous leishmaniasis. *Sci Rep* 2014; **4**: 4156.

**9** Pachioni Jde A, Magalhaes JG, Lima EJ *et al*. Alkylphospholipids—a promising class of chemotherapeutic agents with a broad pharmacological spectrum. *J Pharm Pharm Sci* 2013; **16**: 742–59.

**10** Maes L, Vanden Berghe D, Germonprez N *et al*. *In vitro* and in *vivo* activities of a triterpenoid saponin extract (PX-6518) from the plant *Maesa balansae* against visceral leishmania species. *Antimicrob Agents Chemother* 2004; **48**: 130–6.

**11** Maes L, Cos P, Croft S. The relevance of susceptibility tests, breakpoints and markers. In: Ponte-Sucre A, Diaz E, Padrón-Nieve M, eds. *Drug Resistance in Leishmania Parasites*. London: Springer, 2013; 407–29.

**12** Ménez C, Buyse M, Chacun H *et al*. Modulation of intestinal barrier properties by miltefosine. *Biochem Pharmacol* 2006; **71**: 486–96.

**13** Ménez C, Buyse M, Farinotti R *et al*. Inward translocation of the phospholipid analogue miltefosine across Caco-2 cell membranes exhibits characteristics of a carrier-mediated process. *Lipids* 2007; **42**: 229–40.

**14** Sindermann H, Engel J. Development of miltefosine as an oral treatment for leishmaniasis. *Trans R Soc Trop Med Hyg* 2006; **100** Suppl 1: S17–20.

**15** Reagan-Shaw S, Nihal M, Ahmad N. Dose translation from animal to human studies revisited. *FASEB J* 2007; **22**: 659–61.

**16** 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.