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BIOEQUIVALENCE STUDY OF LUMEFANTRINE RECTAL ENEMA AND THE COMMERCIALLY AVAILABLE GENERIC ORAL SUSPENSION. A PILOT STUDY

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ABSTRACT: Children under five years of age, with severe or cerebral malaria, cannot consume oral medication especially if they are vomiting or are unconscious. In such situations they are given an injectable drug until they can tolerate oral medication. The situation is bad in Sub-Saharan Africa, especially in rural areas as children are sometimes referred to the closest referral health care facility for proper management. The aim of this study was therefore to conduct a pilot study to estimate the bioavailability of lumefantrine (LF) when administered as a rectal enema, and compare it with a commercially available oral suspension, in rabbits. The study was conducted on six healthy rabbits in an open randomized, crossover three sequence, single dose study, where each rabbit received rectal and oral formulations. The oral formulation was administered under fed and fasted conditions. A two-week washout period was allowed between the experiments. LF was quantified in rabbit plasma using ultraperformance liquid chromatography tandem mass spectrometry (UPLC-MS/MS). Results showed that the relative bioavailability of rectal LF was about four times higher than oral. The observed data suggest that a significant adjustment in the dose will be required when LF is administered via the rectal route. The data provide important information for the next step in finding a method to provide a rescue treatment for children with severe or cerebral malaria.

INTRODUCTION: Malaria is a global burden with high mortality reported in children in Sub-Saharan Africa [1-3]. In severe malaria, children can become anemic, have decreased levels of glucose, or cerebral malaria, just to mention a few.

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The susceptibility to other infections increases in these children and they can even develop recurring malaria [4, 5]. It is reported that about US$ 3.1 billion was spent globally due to malaria in 2018 [3, 6].

The World Health Organization (WHO) recommends the use of an artemisinin-based combination therapy (ACTs) for treatment of Plasmodium falciparum malaria. Artemether (AR) and lumefantrine (LF) combination product is one of the ACTs that has been approved for treating uncomplicated *P. falciparum* malaria [7, 8]. Due to

the development of drug resistant malaria parasite, a combination product is the best way to fight such a risk.

AR is derived from artemisinin and works by inhibiting synthesis of proteins in the parasites through production of free radicals [9, 10]. AR has fast onset of action and a short half-life of about (2-3 hours) [9]. It is available as an oral fixed dose combination with LF or as monotherapy dissolved in oil, that is given intramuscularly (IM).

LF belongs to the class of antimalarials called arylamine alcohol [11]. It kills parasites by destroying the hemoglobin in the parasites [9, 10]. Absorption of LF is highly dependent on intake of a fatty meal and may have a lag time of up to 2 hours [9, 12]. LF acts slowly, highest plasma concentration is obtained after 6-8 hours; and has a long elimination half-life of about 2-3 days in healthy human subjects and 4-6 days in *P. falciparum* infected patients [13]. Both AR and LF are highly protein bound i.e., 95.4 % and 99.7 % respectively [11, 13].

Rectal administration of artemisinin derivatives has been reported to achieve acceptable therapeutic outcomes including in severe disease [14]. Currently products available to treat severe falciparum or cerebral malaria contain one drug only in the form of injectables. Children with severe falciparum malaria are given injections of either artesunate, AR or quinine depending on which one of the three monotherapy injectables is available until they can tolerate oral ACTs. When using injections, AR IM is preferred to quinine [15]. In absence of these injectables, especially at the primary level of health care, children below 6 years of age are given rectal artesunate and referred to the next level of health care [14].

However, rectal artesunate is a monotherapy. Due to an increase in emergence of drug resistant malaria parasites, there is a need for a rescue combination product. Although combination products for treating malaria are available, they cannot be used in unconscious and vomiting children. Suppositories for combination antimalarial products have been marketed but are not suitable in the hot temperatures of some Sub-Saharan countries. In addition, the limited volume of fluid in the rectum makes it a challenge as the suppository must either melt or dissolve depending on the nature of the suppository base before the drugs are released into the rectal fluid. Therefore, if a liquid rectal drug delivery system containing ACTs could be made available, that formulation could be an alternative in these children.

Drugs administered rectally can partially bypass metabolism in the liver when the dosage form is inserted on the lower part of the rectum [16]. There have been challenges in solubilizing lumefantrine due to its very poor solubility. A combination dosage form such as a rectal enema, containing both drugs, to be used as an emergency rescue treatment of children that have malaria was recently developed. The aim of this study was therefore to conduct a pilot study to investigate the absorption of lumefantrine when administered as a rectal oily solution, and compare it with a commercially available oral suspension, in rabbits.

When evaluating the bioavailability of a new formulation the reference product is an IV formulation. In certain situations, however, relative, or comparative bioavailability is used, such as when the drug is insoluble and cannot be administered as an IV. In such situations both the reference and test formulations are extravascular products. LF is such a drug as it is not available as an injectable formulation. Lumefantrine is highly dependent on food intake, probably due to the augmentation of the bile salt production, since it has been shown that the relative bioavailability is found to be increased by 16-fold when administered with a high fat diet [12, 17].

METHODS:

Test Materials: Lonart® 20/120 (Bliss GVS Pharma Ltd, India) powder for oral suspension was kindly provided by a local community pharmacy in Malawi. The rectal enema was prepared at the Faculty of Pharmaceutical Sciences at the University of Iceland and contained 8.7 mg artemether and 52 mg lumefantrine per mL. The oral powder was reconstituted with water to make a suspension before administration while the rectal enema was a preformulated formulation that contained AR-LF dissolved in a lipophilic solvent. Both drugs were kindly provided by Mangalam Drugs and Organics Limited (Mumbai, India).

Animals: The study was conducted at the Department of Pharmacology, Faculty of Veterinary Medicine at the University of Murcia, Spain, between the months of May – July 2022. It was approved by the Bioethical Committee of the University of Murcia, Spain (approved experimental protocol number (os 811/2022) and was carried out according to the National Institutes of Health (NIH) guidelines for the care and use of laboratory animals. All efforts were undertaken according to the "3R principles" to reduce the number of animals used in the study and optimize experimental protocols for obtaining maximum data from each tested animal. A limited number of rabbits were used since this was a pilot study to investigate LF absorption from the rectal solution.

New Zealand White (NZW) male and female rabbits were purchased from Granja San Bernardo (Spain). The materials used for the animal experiments consisted of 1.8 mL Eppendorf tubes, amber colored glass vials (2 mL), 22G catheters, 2.5 mL syringes, normal saline (0.9 % saline), alcohol 96 %, were purchased from Proquilab (Murcia, Spain), heparin 1000 IU/mL and female catheters were purchased from Aleman Pharmacy (Murcia, Spain) weighing scales were purchased from Mettler Toledo (Barcelona, Spain), Vortex and mini spin centrifuge were purchased from Merck Life Science (Madrid, Spain).

Study design: A total of six healthy NZW rabbits weighing between 3.75 to 5.14 kg and aged 1-2 years old were housed in individual cages with free access to food and water *at libitum* and maintained on a 12/12 h light /dark cycle at controlled and fixed temperature and humidity at the laboratory animal care facility at the Faculty of Veterinary Medicine at the University of Murcia, Spain. Before the experiments, all animals were allowed to acclimatize for several days.

The study was an open randomized, three sequence single dose crossover study. The animals were weighed and randomized into three groups. In each experiment animals were randomized to receive either: (a) the oral suspension under fasted state; (b) or oral formulation under fed state; (c) or the rectal formulation. The animals that received the oral formulation under fasted state were starved 12 hours before the study but had free access to water. There was a two-week washout period between each experimental sequence. Each rabbit was dosed with the corresponding volume of either the oral suspension or rectal enema equivalent to 24 mg/kg of LF per administration. All the animals were observed daily for 14 days, after the dosing, for any possible adverse events.

Blood Sampling: A 22G catheter attached to a 2.5 mL syringe was used to collect blood samples (about 1.2 mL) from the marginal ear vein of each rabbit before and after dose administration at the following time intervals: 0, 30, 45, 60 and 90 min, then after 2, 4, 6, 8, 10, 24, 48, and 96 hours**.** Considering the 13 samples that were taken, the total volume did not exceed 10% of the total maximum volume of blood that could be collected without significant changes in apparent volume of distribution and was therefore adequate.Syringes (2.5 mL) were flushed/rinsed with approximately 0.2 mL heparin (1000 IU/ mL) and used to collect blood samples into appropriately labelled 1.8 mL transparent Eppendorf tubes.

Blood samples were immediately centrifuged on a mini spin Eppendorf at 12,000 rpm for 10 min. Plasma was collected and divided into two amber colored glass vials (2 mL) and put in a freezer at -80 ± 3°C. At the end of the study, samples were packed and shipped to Iceland for analysis, using *reusable iceless containers* provided by World Courier® shipping company. Upon arrival in Iceland the samples were stored in a freezer at -80 ± 3°C until analysis.

Preparation of Standard Solutions: Stock solution of LF was prepared in MeOH with 0.5 % formic acid. The internal standard (IS), Lumefantrine-d9 was dissolved in MeOH with 0.5% formic acid. All stock solutions were stored at -20°C until use. Working solutions containing LF were prepared using serial dilution with MeOH: water (50:50 v/v).

Eight calibration samples and three quality control (QC) samples were prepared by spiking blank human plasma with 10 µL of appropriate working solution to 90 µL of blank plasma to create standards and quality control samples. The concentration of the calibration samples in plasma were 5, 10, 20, 50, 250, 500, 2000 and 5000 ng/ml

for LF. QC samples in plasm were 15, 150 and 3000 ng/mL.

Plasma Sample Preparation: Rabbit plasma sample (100µL) was added to a 96 well plate followed by 300 µL of MeOH + 0.5% acetic acid containing IS, plate was sealed with cap mat and samples vortexed for 1 min at 1050 RPM. Samples were then centrifuged at 2500 g for 10 min. The supernatant (200 µL) was transferred into a new 96 well plate. The sample (2 µL) was injected into the UPLC-MS/MS system. The limit of quantification (LOQ) was 5 ng/mL.

UPLC_MS/MS Method for Quantification of Lumefantrine: The quantification of LF was performed on a Waters Acquity I-Class UPLC system, coupled to a Waters Xevo TQ-XS triple quadrupole mass spectrometer equipped with electrospray ionization (ESI) probe. Nitrogen was used as a desolvation and cone gas and high purity argon as a collision gas. Source temperature was set to 150°C and desolvation gas temperature was 600°C,at a flow rate of 1000 L h^{-1}; cone gas flow rate 150 L h^{-1}.

The analytical column, Acquity Premier BEH (1.7 µm, 50 × 2.1 mm) (Waters Corporation, Wexford, Ireland), was maintained at 30 °C. The injection volume was 2.0 µL and the sample manager temperature was maintained at ambient temperature. The gradient system consisted of mobile phase A: 0.5% formic acid in water and mobile phase B: 0.5% formic acid in methanol, at a flow rate of 0.50 mL/min.

Initial conditions starting at 32% mobile phase A followed by a linear gradient to 88% of mobile phase B at 2.0 min then back to initial conditions at 2.1 min and then held for 1.9 min. The total chromatographic run time was 4.0 min and the retention time of lumefantrine was 1.6 min. The mass spectrometer was optimized for analyzing LF and lumefantrine-d9 using multiple-reactions monitoring in the positive ESI mode to monitor precursor ion → product ion m/z 527.95 → 509.92 for lumefantrine and 538.90 → 521.03 for lumefantrine-d9. Lumefantrine-d9 was used as an internal standard for LF.

Pharmacokinetic Calculations and Statistical Analysis: Non compartmental analysis (NCA) was used to determine the PK profile of LF using PK Solver 2.0, an Add–in software for Microsoft Excel and Pkanalix 2021R2 (Lixoft SAS, a Simulations Plus Company). Microsoft® Excel® for Microsoft 365 was used for calculations. QI Macros 2023 an Add-in program for Microsoft Excel was used for statistical analysis. Nonparametric tests i.e., Kruskal-Wallis test was used to determine whether a significant difference among the median of three groups existed or not ($\alpha = 0.05$).

The Mann Whitney test was used to compare the medians of two groups using the Bonferroni correction to decrease the probability of committing a type 1 error. The Holm – Bonferroni method was used to decrease the probability of committing a type 2 error since the single step Bonferroni lack statistical power. A t-test was also used to detect the presence or absence of a significant difference between the means of two groups.

The $C_{max}$and t_{max}were obtained directly from the plasma concentration time data by PK Solver and Pkanalix software. The area under the plasma time curve (AUC) from time zero to the last observed time was calculated using the linear trapezoidal method by the Software. The AUC, extrapolated from the last data point to infinity, was estimated by dividing the concentration at the last time point by the terminal elimination rate constant using PK Solver and PKanalix.

Equation 1 was used to calculate relative bioavailability.

$$F = AUC_{test}\ (0\text{-}96\ h) \times D_{reference} / AUC_{oral}\ (0\text{-}96\ h \times D_{test} \quad \text{..(Eq 1)}$$

Where F is the bioavailability, AUC0-96h is the area under the curve for the test formulation (rectal or oral fasted), and the reference formulation (oral fed); and D is the dose for same formulations. Total body clearance was calculated using Equation 2 and apparent plasma clearance using Equation 3:

$$Cl = k_e \times V_d \quad \text{..... (Eq 2)}$$

$$Cl/F = D / AUC_{0\text{-}\infty} \quad \text{..... (Eq 3)}$$

Where Cl is the clearance and Cl/F is the apparent plasma clearance of drug after extravascular administration. The volume of distribution (Vd)

and the apparent volumeof distribution (Vd/F) was calculated using Equation 4 and 5:

$$V_d = D/C_{max} \quad \text{(Eq 4)}$$

$$V_d/F = Cl/F/k_e \quad \text{(Eq 5)}$$

Where Cmax is the maximal plasma concentration and ke is the elimination rate constant. AUC from last observed data to infinity was calculated using Equation 6:

$$AUC_{96-\infty} = C_{last} / k_e \quad \text{(Eq 6)}$$

Where Clast represents the last observed concentration, after 96 hours.

RESULTS: Both the reference and test formulations seemed to be well tolerated in the animals, as no signs indicated discomfort or irritation. Six rabbits participated in the study. Leakage of the rectal formulation occurred in a female rabbit a few minutes after administration and, therefore, it was decided to repeat the rectal formulation and skip the oral fasted sequence. The relative bioavailability (F) of LF calculated from time zero to 96h time point following rectal administration with respect to the oral administration dosed under fed state was 4.18 (418%) as shown in **Table 1**. The Table shows the pharmacokinetic parameters following oral and rectal administration of LF. The relative bioavailability of the rectal enema with respect to the oral administration dosed under fasted state was 4.50 (450%). The maximal plasma concentration following rectal administration was found to be 1,924 ng/mL, compared to 883 ng/mL following oral (fed) animals.

The elimination rate constant, however, was comparable for all animals. The t_{max} was long for oral administration as expected or about 6 -10 hours. However, for the rectal group the t_{max} was found to be unusually long, or about 14.6 hours. For LF, Kruskal-Wallis test showed that a significant difference ($\alpha < 0.05$) existed among treatment groups, both with respect to C_{max} and AUC. Post hoc analysis using the adjusted Holm-Bonferroni α-value revealed that the difference was only significant between the oral groups and the rectal group, but no significant difference was found between the two oral groups.

TABLE 1: THE PHARMACOKINETIC PARAMETERS OF LUMEFANTRINE, FOLLOWING ORAL (FASTED OR FED) ADMINISTRATION OF THE PRODUCT LONART® 20/120 SUSPENSION, AND COMPARED WITH RECTAL ADMINISTRATION OF LF. ALL ANIMALS RECEIVED 24 MG LF PER KG BODY WEIGHT. SIGNIFICANCE WAS MEASURED BETWEEN ORAL (FED) ANIMALS AND RECTAL ADMINISTRATION

	Rectal	Oral (fed)	Oral (fasted)	*p*-value [1]
D (mg/kg)	**24**	**24**	**24**	
C_{max} (ng/mL)	1,924 ± 658	883 ± 471	333 ± 301	0.012
t_{max} (h)	14.6 ± 7.2	6.0 ± 1.3	10.0 ± 8.1	0.077
V_d/F (L/kg)	14 ± 4	45 ± 48	82 ± 73	0.167
V_d (L)	272 ± 204	198 ± 195	359 ± 243	0.537
k_e (h^{-1})	0.030 ± 0.017	0.037 ± 0.014	0.044 ± 0.020	0.465
$t_{½}$ (h)	33.8 ± 25.1	24.3 ± 18.9	18.4 ± 7.7	0.475
MRT (h)	48.0 ± 31.8	31.1 ± 21.0	33.2 ± 6.5	0.306
Cl/F (L/h/kg)	0.36 ± 0.15	1.6 ± 1.9	4.6 ± 2.9	0.162
Cl(L/h/kg)	1.4 ± 1.1	1.6 ± 1.9	3.0 ± 1.7	0.840
AUC_{0-96h} (ng/mL·h)	53,501 ± 26,404	16,815 ± 10,426	11,576 ± 9,986	0.008
AUC_{0-inf} (ng/mL·h)	63,861 ± 37,445	17,575 ± 10,186	11,893 ± 10,087	0.027
F_{rel}	4.18 ± 2.73	(Reference value)	0.58 ± 0.282[2]	

1 = comparing oral (fed) v.s. rectal administration; 2 = (n = 4) because an outlier was removed

When the median peak times were analyzed instead of average, the t_{max} was found to be 6 h (4–8 h), 6 h (4–24 h) and 10 h (10-24 h) following oral fed, oral fasted and rectal administration, respectively. No significant difference was found for the half-lives of LF. Similarly, the median weight adjusted apparent clearance (*Cl/F*) was found to be 0.85 L/h/kg, 2.33 L/h/kg and 1.12 L/h/kg following oral fed, oral fasted, and rectal administration respectively. The median weight adjusted apparent volume of distribution (V_d/F) was found to be 27 L/kg, 71 L/kg, and 47 L/kg, for oral fed, oral fasted and rectal administration, respectively. The median values for the mean residence time (MRT) for oral fed, oral fasted and rectal administration were 25h, 31h, and 36h respectively.

Fig. 1 shows the average plasma concentration time curve following oral fed, oral fasted and rectal administration to rabbits. The amount of LF absorbed following rectal administration of LF in a lipophilic vehicle was found to increase the amount absorbed over 4-5-fold (up to 9.4-fold) compared to oral administration.

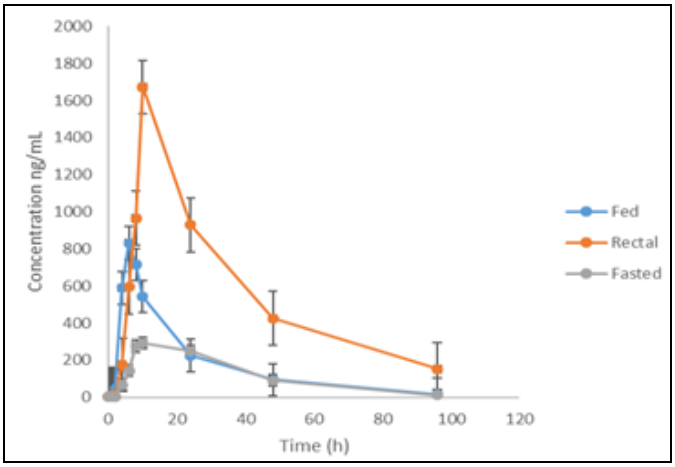

FIG. 1: MEAN PLASMA CONCENTRATION TIME PROFILE FOR LUMEFANTRINE FOLLOWING ADMINISTRATION OF LONART® 20/120 TO HEALTHY RABBITS. BLUE, ORAL DELIVERY TO FED RABBITS; GRAY, ORAL DELIVERY TO FASTED RABBITS; AND ORANGE, RECTAL ADMINISTRATION OF THE ENEMA. ERROR BARS REPRESENT STANDARD ERROR

DISCUSSION: Findings from this pilot study indicate that the relative bioavailability of LF following rectal administration was at least four times higher than following oral suspension. When compared to fasted animals, the bioavailability was found to be even higher or ≥6 fold indicating that rectal administration was superior to oral delivery, and that access to food seems to be important for oral administration, since the bioavailability drops down to 0.58 (when one outlier is eliminated) in animals that were not allowed to eat 12 h prior to the study.

If these data reflect a real clinical situation, a 120 mg dose of LF could be reduced to about 30 mg. If the pediatric dose of 120 mg is calculated based on the fact that children that cannot be fed, should get enough drug, then it should be possible to reduce the dose down to about 17 mg [18]. To follow up on this work, it will be necessary to recalculate the clinical dose required [18]. Although rectally administered drugs partially bypass the first pass metabolism, this does not explain the difference observed in the bioavailability. Especially when other pharmacokinetic parameters are studied, such as t_{max}. Orally administered LF requires bile salts to become solubilized, and absorbed, this explains the difference between oral fasted and oral fed bioavailability data. If bile salts are limited, for example when a child does not have access to food, or if the child is unconscious or cannot take food orally, the bioavailability will be lower or even negligible. Formulations, especially bio adhesive formulation, that can keep LF in a soluble form throughout the study may explain current findings.

Clinical data have shown even more differences between fasted and fed individuals receiving LF [12, 13] although our data does not show a significant difference. One possible reason could be that 2 h after the study started, the rabbits gained free access to food and water, which may have facilitated secretion of bile salts. Something that would not be the case in disease management situations.

Data in **Table 1** indicates large individual differences which is in line with data reported in literature. Considerable inter individual variability has been reported when LF is administered orally in clinical studies done in human beings[13]. Absorption of drugs following rectal administration is also reported to be erratic and highly variable [16, 19] but still beneficial when they produce desired pharmacokinetic profiles and therefore can be used in situations where patients cannot swallow oral medication [19, 20]. In this pilot study, good information was gathered, and the next step would be to carry out a full-size bioequivalence study for proper statistical analysis, as well as metabolite analysis.

Following oral absorption, a lag time is reported for LF solid dosage forms [21] as the drug must first disintegrate and become dissolved before being absorbed. The observed time to peak plasma concentration (t_{max}) following oral administration in rabbits was similar to that observed in human beings (6–8 h) [22, 23]. Other researchers have also reported peak times of 6h and 8h, respectively, in a bioequivalence study of two generic AR–LF products dosed orally in rabbits [24]. However, the case is different with rectal enema since the formulation tested contained dissolved LF in a

lipophilic vehicle. Therefore, the drug bypassed the disintegration and dissolution stages, and the absorption was expected to be rapid. The observed median peak time following rectal administration, however, was found to be 10 h, something that is quite long. This long peak time (t_{max}) may partly be explained by the formulation components/ excipients and the anatomical difference between rabbit rectal anatomy, compared to human rectal anatomy as shown in **Fig. 2.**

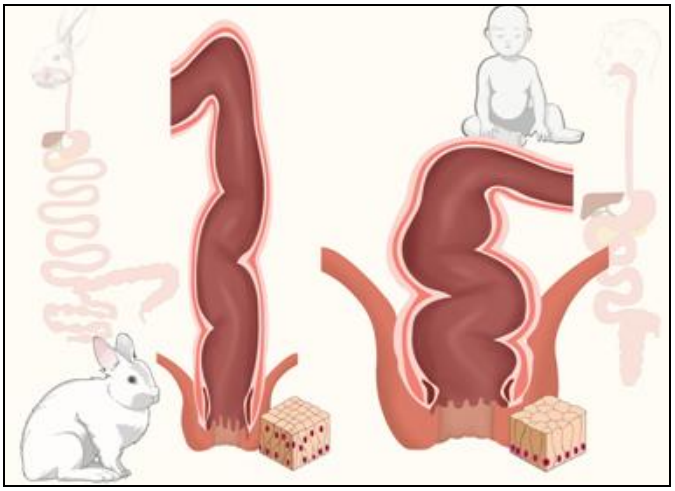

FIG. 2: SHOWING THE ANATOMICAL DIFFERENCES BETWEEN THE RABBIT AND HUMAN RECTUM, INCLUDING THE CELLULAR STRUCTURE INSIDE THE RECTAL MUCOSA

The thickness of the rectal mucosa of rabbits weighing 2 kg was reported to be 280 µm [25] while in humans it is 150 µm [26]. Therefore, the physiology of the rabbit rectal mucosa may be more resistant to allowing compounds such as LF to be transported into the systemic circulation. In addition, human rectal mucosa does not have much water, and the transport rate and transport capacity of LF across the rectal mucosa is not known. Therefore, the long t_{max} observed in rabbits require additional studies, where the difference of human and rabbit rectal anatomy and physiology will be examined [27].

Having a bio adhesive lipophilic formulation may allow the formulation to stick to the rectal mucosa, transporting the drug slowly across the rectal mucosa. This needs to be studied in more detail when human trials will be planned, or in more anatomically similar animal species. Drugs that are absorbed from the rectum may be taken up through three veins: the superior, middle, and inferior hemorrhoidal (rectal) veins. The superior hemorrhoidal vein is connected to the portal venous system, so drugs absorbed into this vein will undergo first pass hepatic metabolism before reaching the systemic circulation. The middle and inferior rectal veins absorb the drug(s) directly into the systemic circulation bypassing the first pass hepatic metabolism [19]. In addition, lymphatic drainage may also happen through the rectal mucosa, which will bypass first pass metabolism, and may end up in the systemic circulation, something that may happen for very lipophilic drugs [16].

A significant difference was found when comparing the C_{max} between the rectal and oral administration groups, showing about 2.2-fold higher C_{max} for the rectal formulation compared to oral (fed) group and 5.8 times higher than in the oral fasted group. Based on this data, it may be required to recalculate the rectal dose, and administration schedule for a possible AR-LF rectal enema.

The mean apparent clearances of LF were found to be around 1.7 L/h, 7 L/h and 21 L/h following rectal, oral fed and fasted states respectively. The clearance will be important to determine the maintenance dose and frequency of drug administration[28]. Because of the long t_{max}, the distribution phase could not be studied and the concentration in different organs was not part of this study but could be an interesting future study[29, 30].

No significant difference was found for the half-lives of LF, indicating similar metabolisms for oral and rectal routes. The median half-life ($t_{½}$) for LF in this study was found to be about 17 h and the average half-life of 24 h when dosed orally under fed state, while in healthy human individuals the reported half-life is from 48-72h. The metabolic rate in rabbits may be faster than in humans, giving a shorter half-life. The median and average half-lives following rectal administration were 22 h and 34 h respectively.

Oral absorption of LF is affected by multiple factors, that will affect the pharmacokinetics of this drug, and need to be considered. It has been reported that LF absorption may rise by 5-fold, when taken with soy milk and16 fold when taken

with a fatty meal [12], since bile salts are important to ensure complete solubilization. A formulation that does not require fatty food intake prior to drug administration, is, therefore, very important when treating critically ill patients with severe or cerebral malaria. Therefore, rectal enema could be useful in such situations.

CONCLUSIONS: AR-LF oral suspension and rectal enema were successfully administered to healthy rabbits and compared. The bioavailability of LF showed that a significantly higher bioavailability was found following rectal administration, compared with the oral route. The t_{max}, however, was surprisingly long and required some additional studies, especially with respect to the anatomical and physiological difference between rabbits and humans.

The observed data suggest that a significant adjustment in the dose will be required when LF is administered via the rectal route, to receive comparable plasma levels as for healthy adults. Although this pilot study had low power, due to the small sample size, the data provide important information for the next step in finding a method to provide a rescue treatment for children with severe or cerebral malaria. A clinical study with high power should be conducted.

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Institutional Review Board Statement: Not applicable.

Informed Consent Statement: The study was approved by the Bioethical Committee of the University of Murcia, Spain (approved experimental protocol number os 811/2022).

Patents: E.K.G.M, B.R.S and S.G. filed a patent application back in 2019 on the dissolution of lumefantrine and artemether.

Data Availability Statement: N/A

CONFLICTS OF INTEREST: The authors declare no conflict of interest.

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