Cerebral uptake of mefloquine enantiomers with and without the P-gp inhibitor elacridar (GF1210918) in mice

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Objectives

To determine whether there is a difference in brain transport between the two enantiomers of mefloquine after administration of the racemic mixture in mice

To investigate the consequences of the combination with the efflux protein inhibitor elacridar on the brain transport of the two enantiomers

Introduction

Mefloquine is a chiral neurotoxic antimalarial agent showing stereoselective brain uptake in humans and rats. Both enantiomers show similar antimalarial activity against *Plasmodium falciparum*, but the specific neurotoxic effect of separated enantiomers is not known. Mefloquine is a substrate and an inhibitor of the efflux protein P-glycoprotein.

We investigated the stereoselective uptake and efflux of mefloquine in mice, and the consequences of the combination with an efflux protein inhibitor, elacridar (GF120918) on its brain transport.

Data and Methods

Chemicals:

- Racemic mefloquine (Hoffmann La Roche, Basel, Switzerland), denoted MQ in the tables and figures.
- P-gp inhibitor GF120918 (elacridar, Glaxo Smith Kline, Marly-le-Roi, France): suspended in a PEG600 / water (25/75, v/v) mixture to a final concentration of 1.5 mg.L^{-1}.

Study design:

- Two groups of OF1 mice
  - group A: repeated intraperitoneal injections of 220 μg of elacridar [100 μL of a 2.2 mg.L^{-1} solution of elacridar suspended in a PEG600 / water (25/75, v/v)]
  - group B: 100 μL of a placebo [PEG600 / water (25/75, v/v)]
  - elacridar or placebo injected 20 min prior to mefloquine injection and repeated twice daily until sacrifice
- Pharmacokinetics of mefloquine studied following a single intraperitoneal injection of 586 μg of racemic mefloquine [100 μL of a 5.86 mg/mL solution of mefloquine solubilised in PEG600 / water (25/75, v/v)]
  - 6 to 7 mice sacrificed at each of the following times after mefloquine treatment: 30 min, 1, 2, 5, 8, 17, 24, 48, 72, 120, and 168 hours
  - brain and whole blood samples collected after sacrifice
  - samples frozen at -20°C until HPLC analysis

Experiments were conducted according to the *'Guidance on the Operation of the Animals (Scientific Procedures) Act 1986'*.

Analytical methods:

- Liquid chromatographic equipment
  - WISP 717+ automatic sample injector
  - Shimadzu LC10 AV pump
  - SPD10 AV spectrophotometric detector
  - Class VP automated software system
- Mefloquine enantiomers determined using a sequential achiral-chiral chromatography
  - achiral column: Lichrospher 100 RP-18 (5 μm) (Lichrocart 125-4 HPLC cartridge) guard column, mobile phase: acetonitrile/ water (50/50, v/v) modified with orthophosphoric acid (400 μl.L^{-1}) and diethylamine (80 μl.L^{-1})
  - chiral column: 150 x 4.6 mm Ultron ES-OVM ovomucoid, mobile phase: acetonitrile/ 20 mM pH 5.8 phosphate buffer (20/80, v/v)
- Analyses performed at room temperature and at a flow rate of 1.0 mL.min^{-1} and at 285 nm and 230 nm for the achiral and chiral chromatographies, respectively

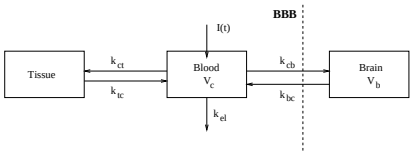
Statistical analysis:

- Building of a joint model to describe the pharmacokinetics of the two enantiomers of mefloquine in blood and brain:
  - comparison of two-, three- and four-compartment models
  - absorption and elimination from the blood compartment
- Statistical methods
  - naive pooling of data (NPD) approach
  - non linear regression weighted by the empirical variance
  - model comparison using log-likelihood ratio tests (LRT) and the Akaike criterion
  - software: R, with library nls2
- Modelling of the pharmacokinetic parameters
  - θ: parameters for (+)mefloquine in the group without elacridar (reference)
  - $\theta_k^- = \theta_k^+ \times \alpha_k$: component θ_k^- of the vector of parameters for (-)mefloquine in the same group
  - $\theta_{GG,k}^+ = \theta_k^+ \times \beta_k$: component θ_k^- of the vector of parameters for (+)mefloquine in the group with elacridar
  - $\theta_{GG,k}^- = \theta_k^+ \times \alpha_k \times \beta_k \times \gamma_k = \theta_k^- \times \beta_k \times \gamma_k$: component θ_k^- of the vector of parameters for (-)mefloquine in the group with elacridar
- Comparison of the pharmacokinetic parameters between the two enantiomers and between the two groups
  - iterative backward procedure starting from full model (all α different from 1)
  - test each α_k one at a time using LRT
  - remove α_k with smallest difference in log-likelihood
  - repeat same procedure with the β and γ

Results

Model building:

Figure 1: A three-compartment model with zero-order absorption from the injection site was found to best represent the pharmacokinetics of both enantiomers in blood and brain.



Model equations:

$$\begin{aligned}
\frac{dC_c}{dt} &= I(t) + k_{bc}\frac{V_b}{V_c}C_b + \frac{k_{tc}}{V_c}Q_t - (k_{el} + k_{cb} + k_{ct})\,C_c \\
\frac{dC_b}{dt} &= k_{cb}\frac{V_c}{V_b}C_c - k_{bc}\,C_b \\
\frac{dQ_t}{dt} &= k_{ct}\,V_c\,C_c - k_{tc}\,Q_t
\end{aligned} \qquad (1)$$

where

- C_c: concentration in the blood (central) compartment for a given enantiomer in one of the group
- C_b: concentration in the brain
- Q_t: amount in the additional tissue compartment
- I(t): input of drug into the central compartment
  - zero-order absorption of duration T_{lag}
  - T_{lag} fixed to 0.01 hr after estimation using grid search
- Pharmacokinetic parameters
  - k_{ct}, k_{tc}: rate constants to and from tissue compartment
  - k_{cb}, k_{bc}: rate constants to and from brain compartment
  - k_{el}: the elimination rate constant
  - V_c, V_b: apparent volumes of distribution in central and brain compartment

Model fit:

Figure 2: predicted and observed concentration versus time profiles for both enantiomers in blood, without (left) or with (right) elacridar. The administration of elacridar did not change blood concentrations of the two enantiomers.

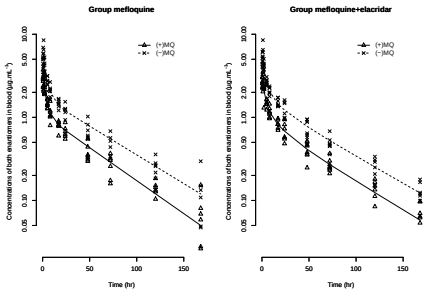
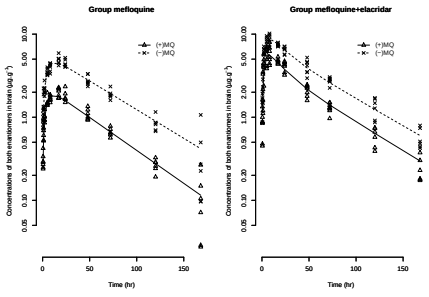


Figure 3: predicted and observed concentration versus time profiles for both enantiomers in brain, without (left) or with (right) elacridar. After administration of elacridar, brain concentrations of the two enantiomers increased.



- good fit for both enantiomers in the two organs
- adequacy of the model confirmed using diagnostic plots (not shown)

Influence of efflux inhibition on the pharmacokinetics of mefloquine enantiomers in blood and brain:

Table 1: Estimated parameters (and standard errors of estimation in brackets). The values for (+)mefloquine in the group given mefloquine without the inhibitor are taken as reference. α represents the change for (-)mefloquine relative to the reference. β represents the change for (+)mefloquine after the addition of elacridar, relative to the reference. γ represents the differential effect of elacridar on (-)mefloquine with respect to (+)mefloquine.

	Mefloquine alone		Pre-treatment with elacridar	
Parameter	Value for (+)MQ	α	β	γ
k_{el} (hr^{-1})	0.047 (0.001)	-	-	-
k_{cb} (hr^{-1})	0.127 (0.016)	-	-	-
k_{bc} (hr^{-1})	0.107 (0.004)	0.80 (0.03)	1.62 (0.08)	-
k_{ct} (hr^{-1})	0.031 (0.008)	-	-	-
k_{tc} (hr^{-1})	0.256 (0.234)	-	0.17 (0.17)	-
V_c/F_c (mL)	122 (3)	0.53 (0.01)	-	-
V_b/F_b (g)	76 (9)	0.44 (0.01)	0.25 (0.01)	1.59 (0.05)

Most of the parameters of the model remained unchanged after pre-treatment with elacridar, but the transfer rate constant from brain to blood increased for both enantiomers, while the transfer rate constant from tissue to blood decreased. The volumes of distribution in brain were lower by 75% for (+)mefloquine and 60% for (-)mefloquine with elacridar (p<0.001 for both).

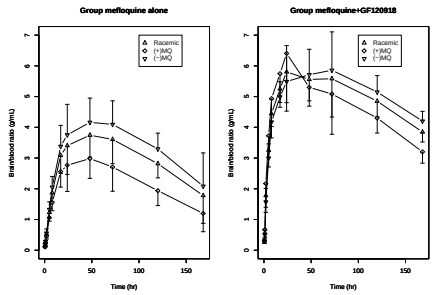
Table 2: Derived parameters estimated for each enantiomer, with or without elacridar.

	Mefloquine alone		Pre-treatment with elacridar	
Parameter	(+)MQ	(-)MQ	(+)MQ	(-)MQ
CL_{cb}/F_c (mL.hr^{-1})	15.5	8.2	15.5	8.2
CL_{bc}/F_b (g.hr^{-1})	8.2	2.9	3.3	1.9
CL_{ct}/F_c (mL.hr^{-1})	5.7	3.6	5.7	3.6
V_c/F_c (mL)	122	65	122	65
V_b/F_b (g)	77	34	19	13
AUC_c (μg.mL^{-1}.hr)	66	104	66	104
AUC_b (μg.g^{-1}.hr)	124	349	307	533

Because the apparent volumes of distribution in the brain decreased, the efflux clearances of both enantiomers decreased in the presence of the inhibitor (respectively by 60 and 35% for the (+) and (-) enantiomers).

Brain uptake of mefloquine enantiomers:

Figure 4: brain/blood concentration ratios of the two enantiomers and the racemic with and without pre-treatment with elacridar.



Enantiomeric ratios are inverted during the first hour of treatment with elacridar, when compared to the figure without elacridar. When comparing areas under the brain/blood ratio curves, brain-blood ratios for both enaintiomers increased after elacridar, to 1.83 and 1.73 respectively for (+) and (-)mefloquine compared to 0.74 and 1.1 in the absence of elacridar. We also found that the stereoselectivity observed in mefloquine brain uptake without elacridar was almost suppressed by the pre-treatment with elacridar.

Conclusion

(-)Mefloquine had a lower blood and brain apparent volume of distribution and a lower efflux clearance from the brain, resulting in a larger brain/blood ratio compared to (+)mefloquine. Elacridar did not modify blood concentrations or the elimination rate from blood for either enantiomers. However, cerebral AUC of both enantiomers were increased after elacridar administration, with a stronger effect on (+)mefloquine.

After administration of racemic mefloquine in mice, blood and brain pharmacokinetics are stereoselective, (+)mefloquine being excreted from brain more rapidly than its antipode, showing that mefloquine is a substrate of efflux proteins and that mefloquine enantiomers undergo efflux in a stereoselective manner. Moreover, pretreatment with elacridar reduced the brain efflux clearances with a more pronounced effect on (+)mefloquine.

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Published in ***British Journal of Clinical Pharmacology****, 2004. Barraud de Lagerie S., Comets E. et al.; 141:1214-1222.*