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Short Communication

Direct determination of the enantiomers of the antimalarial drug halofantrine and its active metabolite N-desbutylhalofantrine in human plasma

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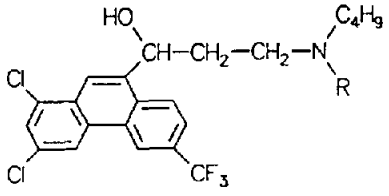
Abstract

The plasma concentrations *vs.* time profiles of the enantiomers of halofantrine and its main metabolite N-desbutylhalofantrine have been determined in two healthy volunteers after a single oral administration of 500 mg of racemic halofantrine hydrochloride (Halfan). A solid-phase extraction method was used for the purification of the samples. The enantiomers of halofantrine and N-desbutylhalofantrine were determined simultaneously using chiral high-performance liquid chromatography on chiral stationary phases with fluorescence detection.

1. Introduction

Halofantrine, *R,S*-1-(1,3-dichloro-6-trifluoromethylphenanthryl)-3-N,N-dibutylaminopropan-1-ol (Fig. 1), a chiral phenanthrene methanol derivative, is an orally administered blood schizontocide, active against both chloroquine-sensitive and chloroquine-resistant plasmodia. The drug is marketed as the racemate. The main metabolite, N-desbutylhalofantrine (Fig. 1), is also pharmacologically active [1,2]. The pharmacokinetics of racemic halofantrine and N-desbutylhalofantrine has been investigated [3,4] and fluorescence detection of halofantrine has been described [5].



R = C_4H_9 : Halofantrine
R = H : N-Desbutylhalofantrine

Fig. 1. Structure of halofantrine and N-desbutylhalofantrine.

Previously we have accomplished the chiral resolution of halofantrine and its metabolite N-desbutylhalofantrine without prior achiral separation on Chiralcel OD and Chiralpak AD columns [6]. Using that HPLC method the biotransformation of halofantrine was investigated *in vitro* in phenobarbitone-induced rat microsomal fractions. (−)-Halofantrine was found to be metabolized preferentially [6].

In the present communication we report the determination of the enantiomers of halofantrine and its metabolite N-desbutylhalofantrine in

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human plasma after oral administration of racemic halofantrine to two healthy volunteers.

2. Experimental

2.1. Apparatus

The chromatographic system consisted of a Merck Hitachi L-6000 pump, a Merck Hitachi F-1050 fluorescence detector operated at an excitation wavelength of 300 nm and an emission wavelength of 380 nm, an automated sample injector (Kontron MSI 660) equipped with a 200-μl loop and a Merck Hitachi D-2500 chromato-integrator.

2.1. Chemicals

Racemic halofantrine hydrochloride and N-desbutylhalofantrine hydrochloride were gifts from SK and F (Welwyn Garden City, UK), the internal standard desipramine hydrochloride was from Ciba Geigy (Wehr, Germany). HPLC solvents were purchased from Merck (Darmstadt, Germany) and 500-mg Bakerbond solid-phase extraction columns were from Baker Chemikalien (Gross-Gerau, Germany). All other chemicals were of analytical grade.

2.3. Chromatographic conditions

The chromatographic separation was achieved on a Chiralpak AD analytical column containing amylose tris-3,5-dimethylphenylcarbamate coated on silica gel (250 × 4.6 mm I.D., 10 μm particle size) equipped with a guard column containing the same material (50 × 4.6 mm I.D., 10 μm particle size) from Baker (Gross-Gerau, Germany). The mobile phase consisted of *n*-hexane–ethanol–2-butanol–diethylamine (93:4.5:2.5:0.1, v/v) operated at a flow-rate of 0.3 ml/min as described previously [6].

2.4. Drug administration and sample collection

Two healthy male volunteers (volunteer A: 30 years of age, 69 kg; volunteer B: 56 years of age, 110 kg) were administered 500 mg (two 250-mg tablets) of halofantrine hydrochloride (Halfan, SK and F). Venous blood samples were collected before and at 2, 7, 9, 11, 24, 28, 32 and 48 h after administration. Blood samples were collected in EDTA coated tubes. The plasma was immediately separated by centrifugation at 2000 *g* for 15 min and stored at −20°C until analysis.

2.5. Extraction of the plasma samples

Frozen plasma samples were allowed to thaw at room temperature for 30 min followed by thorough vortex-mixing. A 1.0-ml volume of each plasma sample was transferred to a siliconized tube and 3 ml of a mixture of acetonitrile–triethylamine–ethanol (99:1:1, v/v) (adjusted to pH 4 with 1 *M* HCl) were added. The solution was applied to a 500-mg C_{18} solid-phase extraction column, which had been preconditioned with 3 × 3 ml of acetonitrile and 3 × 3 ml of the mixture of acetonitrile–triethylamine–ethanol. To assure complete elution of the sample, the cartridge was washed with an additional 2 ml of the solvent mixture. The eluent was collected in a siliconized tube, 5 μl of a solution of the internal standard (10 μg/ml desipramine hydrochloride) were added and the solvent was evaporated under a stream of nitrogen at 40°C. The residue was dissolved in 1 ml of the mobile phase. Aliquots of 200 μl were injected onto the HPLC system.

2.6. Calibration, recovery and reproducibility

Stock solutions of racemic halofantrine hydrochloride (1.00 mg/100 ml), racemic N-desbutylhalofantrine hydrochloride (1.29 mg/100ml) and the internal standard (10 mg/100 ml) were prepared by dissolution in the mobile phase without diethylamine. Calibration curves were constructed from 19 concentration points of spiked plasma samples which contained 5–1000 ng/ml of racemic halofantrine, N-desbutylhalofantrine and 5 ng of the internal standard ($n = 3$).

The recovery of the compounds was estimated using the peak areas obtained from extracted

samples containing known amounts of the compounds.

Intra- and inter-assay reproducibility was assessed by replicate assay of spiked plasma samples with known amounts of the compounds.

3. Results and discussion

Typical chromatograms of plasma samples are shown in Fig 2. No interferences were observed with the peaks of the halofantrine and N-desbutylhalofantrine enantiomers.

The relationship between the concentration and the detector response was determined by injecting a series of halofantrine and N-desbutylhalofantrine with concentrations ranging from 5 to 1000 ng/ml. A logarithmic relationship was observed [5]. The calibration curve was constructed by plotting the logarithm of the ratio of the peak area of each enantiomer to the peak area of the internal standard against the logarithm of the corresponding concentration of the respective enantiomers. A linear regression was used to calculate the slope, the intercept and the correlation coefficient for each calibration curve. The correlation coefficients (r) were for (+)-halofantrine 0.9932, for (−)-halofantrine 0.9965, for the first eluting N-desbutylhalofantrine enantiomer 0.9985, and for the second eluting N-desbutylhalofantrine enantiomer 0.9937.

The slopes were 0.9448 ± 0.093, 0.9682 ± 0.0154, 1.0235 ± 0.0417 and 1.0081 ± 0.0222, the y-intercepts were -1.674 ± 0.043, -1.735 ± 0.031, -1.887 ± 0.029 and -1.8471 ± 0.046, respectively.

The intra- and inter-assay reproducibility and the precision are shown in Table 1. The limit of quantification was at least 6 ng/ml. The recovery is summarized in Table 2.

This method was used to determine the plasma concentrations *vs.* time profiles and the pharmacokinetics of the enantiomers of halofantrine and

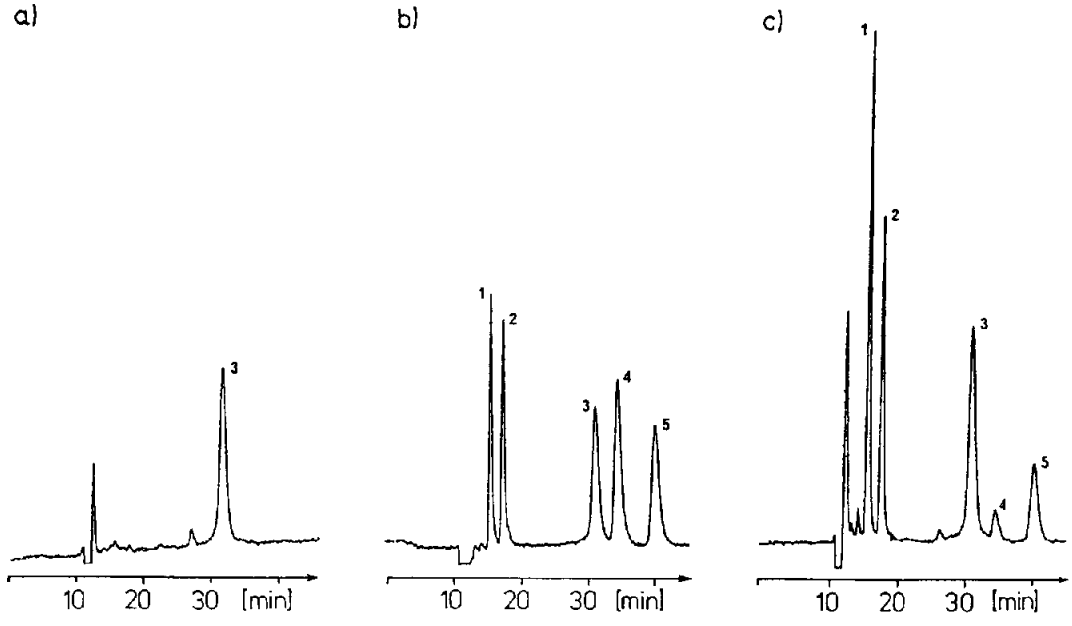

Fig. 2. Chromatograms of plasma samples on ChiralPak AD. (a) Drug free plasma spiked with internal standard. (b) Drug free plasma spiked with internal standard, racemic halofantrine (100 ng/ml) and N-desbutylhalofantrine (129 ng/ml). (c) Plasma sample of volunteer A at 7 h after administration. Peaks: 1 = (+)-halofantrine, 2 = (−)-halofantrine, 3 = internal standard, 4 = N-desbutylhalofantrine enantiomer, metabolite of (−)-halofantrine, 5 = N-desbutylhalofantrine enantiomer, metabolite of (+)-halofantrine. For chromatographic conditions see Experimental.

Table. 1
Reproducibility of the assay

Theoretical concentration (ng/ml)	n	Determined concentration (ng/ml)	S.D. (%)
(+)-Halofantrine			
6.25	5	6.15	9.27
40.5	5	40.8	12.85
150	5	138	3.37
450	5	468	3.2
(−)-Halofantrine			
6.75	5	6.34	15.00
40.7	5	43.7	11.63
150	5	139	2.35
450	5	471	3.74
N-Desbutylhalofantrine (first eluting)			
6.27	5	5.77	1.8
43.9	5	45.5	3.78
212	5	212	0.92
708	5	773	1.14
N-Desbutylhalofantrine (second eluting)			
10.3	5	11.7	10.0
35.9	5	41.1	12.0
174	5	173	0.39
580	5	639	1.56

N-desbutylhalofantrine. The pharmacokinetic variability of the drug in individuals is very important for the treatment of malaria.

Two healthy male volunteers were administered 500 mg of halofantrine hydrochloride as a single oral dose after breakfast. The plasma concentration–time profiles observed for 48 h are presented in Fig. 3.

The plasma concentration–time profiles of

Table 2
Recovery of halofantrine and N-desbutylhalofantrine

Enantiomer	n	Recovery (%) (mean ± S.D.)	R.S.D. (%)
(+)-Halofantrine	3	102 ± 11.28	11
(−)-Halofantrine	3	98.4 ± 3.48	3.54
N-Desbutylhalofantrine (1st)	3	75.9 ± 5.56	7.33
N-Desbutylhalofantrine (2nd)	3	73.9 ± 4.67	6.32

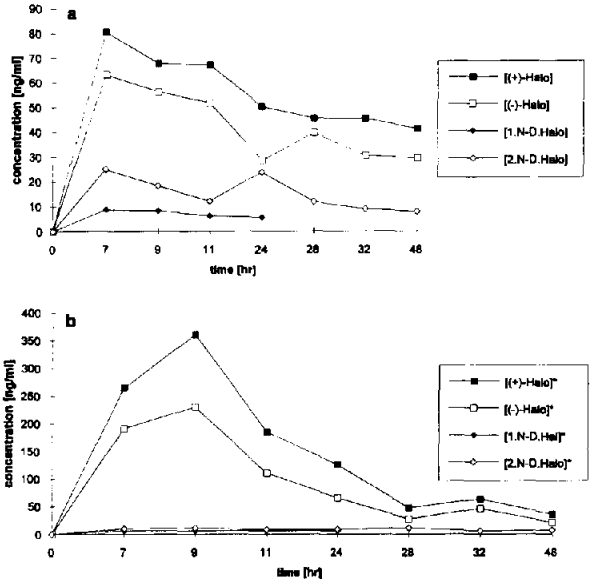

Fig. 3. Concentration–time profile of halofantrine and N-desbutylhalofantrine enantiomers in plasma. (a) Volunteer A, (b) volunteer B.

halofantrine were very different for the two volunteers. The C_{max} values for (+)- and (−)-halofantrine were approximately 80 and 60 ng/ml (volunteer A) and 360 and 210 ng/ml (volunteer B), respectively. The maximum concentrations of the first and second eluting N-desbutylhalofantrine were *ca.* 8 and 25 ng/ml for A and 7 and 11 ng/ml for B, t_{max} for volunteer A and B was *ca.* 7 and 9 h, respectively. The C_{max} of subject B was therefore *ca.* four times higher than that of subject A. This might be due to intra- and inter-subject variability and/or due to the fat content in the food, which has been shown to affect the absorption of halofantrine [1,7]. Volunteer B consumed a fat enriched breakfast prior to administration. On the other hand, the concentration of N-desbutylhalofantrine of volunteer A is slightly higher than that of volunteer B. These differences might be due to a genetic polymorphism of the subjects (extensive/poor metabolizer).

There were significant differences in the concentrations of the enantiomers of halofantrine. However, in both volunteers (+)-halofantrine was always found in a higher concentration than

the (−)-enantiomer, the enantiomeric ratio ranging between 1.3 and 1.7. This implies that the (−)-enantiomer is metabolised and/or excreted preferentially. This correlates well with the *in vitro* metabolism of halofantrine described in our previous publication [6]. A stereoselective biotransformation of halofantrine with similar results using different chromatographic procedures has been described in a pilot pharmacokinetic study with one volunteer [8].

4. Conclusions

The method for the direct determination of the enantiomers of halofantrine and its main metabolite N-desbutylhalofantrine from human plasma described in this contribution proved to be selective, sensitive, reproducible and simple and could be used for the stereoselective drug monitoring of halofantrine.

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