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Short communication

Analysis of the antimalarial drug halofantrine and its major metabolite N-desbutylhalofantrine in human plasma by high performance liquid chromatography

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Abstract

The determination of halofantrine and its major metabolite N-desbutylhalofantrine in human plasma by reversed phase high-pressure liquid chromatography is described. The method involves protein precipitation of plasma samples by acetonitrile followed by basification with sodium hydroxide and subsequent liquid–liquid extraction using hexane-diethyl ether (1:1, v/v). The chromatographic separation was carried out on a C-18 column with a mobile phase consisting of methanol/0.05 M KH_2PO_4 (78:22, v/v) containing 55 mM perchloric acid. Chlorprothixen was used as internal standard. The relative standard deviations of intraday and interday precision for both compounds were less than 7%, the relative standard deviation of the accuracy did not exceed 7.1% at concentrations of 50 and 300 ng/ml. This method is simple, rapid, sensitive and cost effective and was applied to the determination of the pharmacokinetics of halofantrine and N-desbutylhalofantrine in two healthy male volunteers after an oral administration of 500 mg halofantrine. Moreover, the influence of the frequently consumed kolanut on the pharmacokinetics of halofantrine was investigated.
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1. Introduction

Halofantrine (Hf), 1-(1,3-dichloro-6-trifluoromethylphenanthyl)-3-*N*,*N*-dibutylaminopropan-1-ol (Fig. 1), which was first synthesized by Colwell et al. [1] is an effective drug for the treatment of malaria. The compound is often recommended as the drug of choice against infections with chloroquine and/or pyrimethamine resistant *Plasmodium falciparum* strains for which a high incidence of multidrug resistance has been reported [2,3]. The major metabolite N-desbutylhalofantine (DHf, Fig. 1) is also pharmacologically active. Hf is a highly lipophilic drug with erratic bioavailability [4] known to have dose-related cardiotoxic adverse effects in humans [5]. Thus, simple and reliable methods for the quantification of Hf and DHf, especially, in the setting of a hospital are required in order to achieve optimized malaria therapy with the drug.

Several HPLC methods for the determination of Hf and DHf in biological fluids have been reported [6–13]. However, most methods require solid-phase extraction procedures [7–10], which require additional equipment and the supply of solid-phase extraction cartridges. Other methods have the disadvantage of long run times [10] or poor sensitivity [11,12]. The methods by Onyeji et al. [6] and Humberstone et al. [13] are simple and cost-effective but are hampered by the fact that the internal standard is not commercially available. Especially, in the tropics, where malaria is prevalent as a major disease and financial funds and expensive equipment are limited, there is the need to develop a simple, reliable and cost-effective assay for the simultaneous analysis of Hf and DHf. Thus, the present study was conducted in order to develop an HPLC method with UV detection for HF and DHf employing a commercially available compound as internal standard. The applicability of therapeutic drug monitoring is demonstrated.

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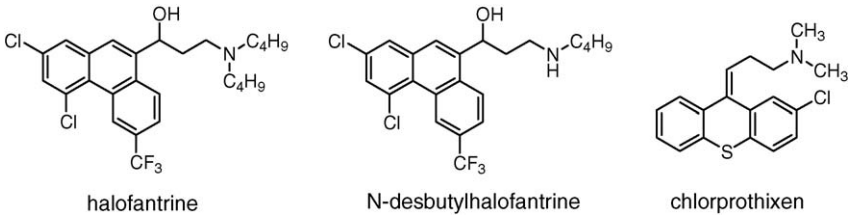

Fig. 1. Structures of halofantrine, N-desbutylhalofantrine and chlorprothixen.

2. Experimental

2.1. Chemicals

Halofantrine hydrochloride and N-desbutylhalofantrine were gifts from SmithKline Beecham (Welwyn Garden City, United Kingdom). Chlorprothixen was purchased from Sigma–Aldrich (Deisenhofen, Germany). HPLC-grade methanol, *n*-hexane and acetonitrile, diethyl ether and potassium dihydrogenphosphate were obtained from VWR International (Darmstadt, Germany). Perchloric acid was purchased from ACROS Organics (Geel, Belgium).

2.2. Chromatographic conditions

Chromatography was performed with a HPLC system consisting of a Shimadzu model LC-10AD pump, a Shimadzu model SPD-10A UV–vis detector and a Shimadzu SCL-10A system control unit. The detector wavelength was set to 254 nm. The Shimadzu Class-VP 5 software was used for data handling. The analytes were separated on a LiChroCART RP-18 column (5 μm, 250 mm × 4 mm i.d.). The mobile phase consisted of methanol/0.05 M KH_2PO_4 (78:22, v/v) containing 55 mM perchloric acid with an apparent pH of 3.1. The flow rate was 1.0 ml/min.

2.3. Extraction procedure

Twenty microliters of a solution of chlorprothixen in methanol (40 μg/ml) were added to 1.0 ml plasma in a 10 ml-stoppered test tube. Two milliliters of acetonitrile were added to precipitate the proteins followed by vortex mixing for 1 min and centrifugation at 4000 rpm for 15 min. The supernatant was then transferred into another 10 ml-stoppered test tube and 1.0 ml of 2 M NaOH and 5.0 ml of *n*-hexane/diethyl ether (1:1, v/v) were added. The mixture was vortexed for 1 min and centrifuged at 4000 rpm for 10 min. 4.5 ml of the organic layer were transferred into a tapered test tube and evaporated to dryness under a stream of nitrogen at 40 °C. The residue was reconstituted in 100 μl of the mobile phase and aliquots of 20 μl were injected into the HPLC.

2.4. Calibration, recovery, precision and accuracy

Stock solutions of Hf and DHf with concentrations between 2.0 and 20.0 μg/ml and a stock solution of chlorprothixen at 40 μg/ml were prepared in methanol. Calibration curves were constructed from six concentration points of spiked plasma containing 20–800 ng/ml Hf and DHf and 800 ng/ml chlorprothixen. The recovery of the compounds was estimated using the peak areas obtained from extracted samples containing known amounts of the compounds and compared to samples prepared in the mobile phase. Intra- and interassay precision was assessed by replicate analysis of spiked plasma samples with known concentrations of the compounds. Accuracy was calculated from the peak areas obtained for the compounds from extracted spiked plasma samples and peak areas of standard solutions.

2.5. Drug administration and sample collection

The study was approved by the University College Hospital Ethical Review Committee at the University of Ibadan with given informed consent from the volunteers. Two healthy male volunteers (age 23 and 26) weighing 52 and 57 kg, respectively, were administered two 250 mg Halfan® tablets (batch 743) as a single oral dose after fasting overnight. Five milliliter-venous blood samples were collected into heparinized tubes before the administration and 1, 2, 3, 4, 6, 8, 12, 24, 48, 72 and 168 h after drug administration. The plasma was immediately separated by centrifugation at 2000 × *g* for 15 min and stored at −20 °C until analyzed.

2.6. Data analysis

The pharmacokinetic parameters were calculated by non-compartmental analysis of the plasma concentration versus time profiles using WinNonlin Standard Edition, Version 1.1 (Scientific Consultant Inc., Apex, USA).

3. Results and discussion

Several HPLC methods for the determination of Hf and DHf have been reported in the literature [6–13]. However, they are hampered by relatively long analysis times, inadequate sensitivity or the fact that the internal standard is not commercially available. Thus, a simple and reliable method was developed based on an earlier publications [6] using chlorprothixen (Fig. 1) as internal standard. Imipramine, which has also previously been employed as internal standard [14] did not completely resolve from plasma constituents in our hands upon liquid–liquid extraction. In addition, a C-18 column was used instead of a C-8 column as it gave superior separation of the analytes from plasma components. Representative chromatograms of blank plasma and spiked plasma

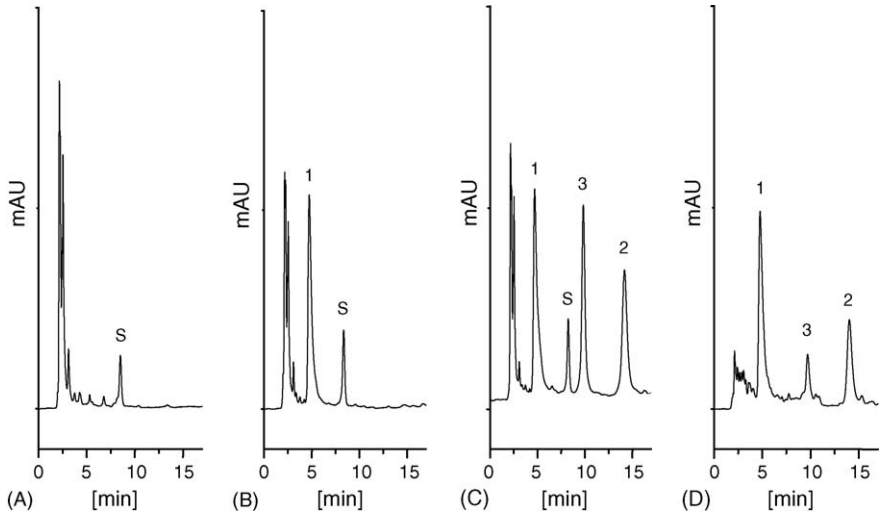

Fig. 2. HPLC chromatograms of: (A) blank human plasma, (B) plasma spiked with the internal standard chlorprothixen, (C) plasma spiked with 300 ng/ml of Hf and DHf as well as 800 ng/ml chlorprothixen, and (D) plasma sample obtained 6 h after a single oral dose of 500 mg Hf hydrochloride to a healthy male volunteer. (1) Chlorprothixen; (2) Hf; (3) DHf; (S) system peak in blank plasma.

at ambient temperature are shown in Fig. 2A–C. A chromatogram of a plasma sample obtained from a healthy volunteer 6 h after oral administration of 500 mg Hf hydrochloride (Halfan® tablets) is shown in Fig. 2D. The assay is complete within 15 min. Antimalarial drugs such as chloroquine, quinine, pyrimethamine, sulfadoxine, and newer antimalarial drugs such as mefloquine and lumefantrine did not interfere with the peaks of Hf, DHf and the internal standard. The very lipophilic drug lumefantrine eluted at 26.6 min, while the other compounds basically coeluted at 2.2–2.3 min with the exception of mefloquine (3.1 min).

Calibration curves for Hf and DHf were linear over a range of 20–800 ng/ml with regression coefficients of at least 0.998. The limit of detection defined at a signal-to-noise ratio 3:1 was 5 ng/ml for both Hf and DHf while the limit of quantitation was 20 ng/ml for both compounds. Precision and accuracy data are summarized in Table 1. Liquid–liquid extraction using hexane/diethyl ether (1:1, v/v) resulted in recoveries from plasma between 85 and 89%. We did not observe additional peaks hinting at a degradation product of DHf as reported by Humberstone et al. [13] when extracting the compounds from alkaline buffer solutions.

The applicability of the method to pharmacokinetic studies was evaluated comparing the pharmacokinetics upon oral administration of 500 mg Hf hydrochloride to two healthy male volunteers after fasting overnight and immediately after administration of 12.5 g kolanut. Kolanut is a habitually consumed fruit in Africa. Fig. 3 illustrates the plasma concentration profiles of HF and DHf over a period of time for the volunteers without (A and B) and with co-administration of kolanut (C and D). The pharmacokinetic data obtained by non-compartmental analysis are summarized in Table 2. The data for the oral administration of 500 mg Hf hydrochloride alone are generally in the range reported in the literature [9,14–19]. For example, Bassi et al. reported values for c_{max} between 190 and 470 ng/ml and for the AUC (0–336 h) between 15.1 and 42.6 μg/ml h [14]. About twice as high c_{max} values were found for volunteer A compared to volunteer B. This may be either caused by the known erratic bioavailability of the drug as both subjects took the tablets on an empty stomach or by genetic polymorphism of the subjects (extensive and poor metabolizer).

Co-administration of kolanut to the volunteers resulted in a significant decrease of c_{max} and AUC values of Hf and DHf compared to the administration of the drug alone (Table 2). Changes in t_{max} and $t_{1/2}$ were small and may be due to the limited number of volunteers. The reason for the altered pharmacokinetics of Hf when co-administered with kolanut is currently unknown. Hf is known to be extensively metabolized by presystemic cytochrome P450 enzymes and, thus, increased plasma concentrations have been observed when the drug is taken with grapefruit juice [15], tetracycline [14] or after pretreatment with ketoconazole [18]. In contrast, decreased plasma concentra-

Table 1
Precision, recovery and accuracy of the analytical method

Drug	*n*	Conc. [ng/ml]	Intraassay precision [CV%]	Interassay precision [CV%]	Recovery mean ± S.D.%	Accuracy mean ± S.D.%
Hf	6	50	5.82	6.59	85.6 ± 3.0	103.2 ± 6.8
	6	300	4.57	4.06	85.4 ± 3.1	100.6 ± 5.7
DHf	6	50	6.97	6.77	89.3 ± 3.3	100.2 ± 7.1
	6	300	5.92	3.86	85.4 ± 2.7	96.6 ± 5.8
Chlorprothixen	6	800			88.8 ± 1.8	

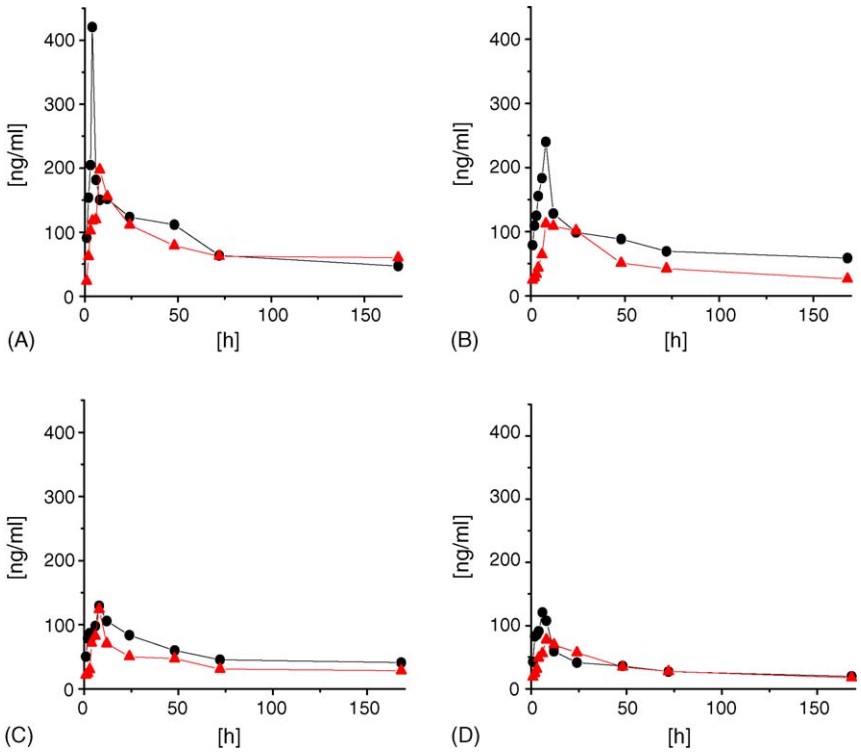

Fig. 3. Plasma concentration vs. time profile of Hf and DHf after oral administration of 500 mg Hf hydrochloride to two healthy male volunteers without (A and B) and with co-administration of 12.5 g kolanut (C and D). (●), Hf; (▲), DHf.

Table 2
Pharmacokinetic parameters of halofrantrine and N-desbutylhalofrantrine after oral administration of 500 mg halofantrine hydrochloride to two healthy male volunteers

Treatment	Volunteer	Compound	c_{max} [ng/ml]	t_{max} [h]	$t_{1/2}$ [h]	AUC (1–168) [μg/ml h]
500 mg Hf HCl	A	Hf	420	4	103	14.10
		DHf	198	8	58	12.97
	B	Hf	240	8	107	13.53
		DHf	112	8	87	8.32
500 mg Hf HCl + 12.5 g kolanut	A	Hf	129	8	54	9.41
		DHf	124	8	57	6.56
	B	Hf	121	6	110	5.58
		DHf	78	8	95	5.39

tions were observed when Hf was co-administered with antacid drugs due to adsorptive effects [19]. It may be hypothesized that kolanut constituents interfere with the absorption of the drug but further studies are required to unequivocally prove this interference. Nevertheless, the present study is another example that co-administration of nutrients and other drugs with Hf may influence the bioavailability of Hf.

4. Conclusion

A simple liquid chromatographic method for the analysis of Hf and its major metabolite DHf in human plasma has been developed and validated using a commercially available compound as internal standard. The method is simple, specific and rapid and, thus, suitable in a relatively simple clinical environment for monitoring Hf and the main metabolite DHf upon oral administration of the drug. Co-administration of kolanut resulted in a decreased bioavailability illustrating that nutrients might influence the oral absorption and/or the metabolism of Hf.

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