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Lack of effect of chloroquine on the debrisoquine (CYP2D6) and S-mephenytoin (CYP2C19) hydroxylation phenotypes

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The effects of chloroquine (CHQ) on debrisoquine hydroxylase (CYP2D6) and S-mephenytoin hydroxylase (CYP2C19) were assessed in 11 black Zimbabwean and 12 white Swedish healthy volunteers. The activity of CYP2D6 was measured as the urinary debrisoquine to 4-hydroxydebrisoquine metabolic ratio and that of CYP2C19 as the urinary S- to R-mephenytoin enantiomer ratio (S/R). There were no statistically significant differences in either metabolic ratio as a result of prophylactic or loading doses of CHQ. This indicates that CHQ does not inhibit CYP2D6 or CYP2C19 *in vivo* and is unlikely to compromise the metabolism of substrates for these two enzymes. It is, therefore, also unlikely that residual CHQ in populations under study will interfere with phenotyping of either CYP2D6 or CYP2C19.

Keywords chloroquine debrisoquine hydroxylase S-mephenytoin hydroxylase phenotyping

Introduction

Cytochromes P450 (CYP) are a super-family of haem proteins responsible for the oxidative metabolism of a wide range of xenobiotics. Of pharmacological importance is the fact that some cytochromes P450 exhibit genetic polymorphisms, including CYP2D6 and CYP2C19 which, between them, metabolize a large number of clinically important drugs [1]. Phenotyping to assign metabolizer status in humans is typically carried out by monitoring the metabolism of a probe drug, such as debrisoquine for CYP2D6 or S/R-mephenytoin for CYP2C19. In Africans, phenotyping studies for CYP2D6 have yielded conflicting results so far [2] whereas CYP2C19 phenotyping has just been initiated [3, 4]. Many explanations have been proposed to account for the inconsistent CYP2D6 phenotyping results in Africans including genetic and environmental factors. Drugs or dietary compounds may inhibit CYP2D6 [5] leading to misclassification of extensive metabolizers (EMs) as poor metabolizers (PMs). Chloroquine (CHQ), widely used in malaria-endemic countries, has been proposed as such a drug since it has been found to inhibit CYP2D6 catalysed reactions both *in vivo* (in rats) and *in vitro* (in human liver microsomes) [6, 7]. This study was, therefore, conducted to assess the effect of chloroquine on CYP2D6 and CYP2C19 metabolic ratios in human subjects.

Methods

Subjects

Black Zimbabwean (11 males, mean age: 27 ± 4 years and 60.8 ± 5.6 kg weight) and white Swedish (nine females and three males, mean age: 38 ± 8 years and 70.2 ± 13 kg weight) subjects participated in this study. None took medications for at least 1 week before and during the study. No evidence of chloroquine could be found in any urine by h.p.l.c. analysis [8] prior to treatment. Subjects were considered healthy as determined by medical history, physical examination and standard laboratory indices. The study was approved by the Ethics Committee of Karolinska Institute and the Medical Research Council of Zimbabwe.

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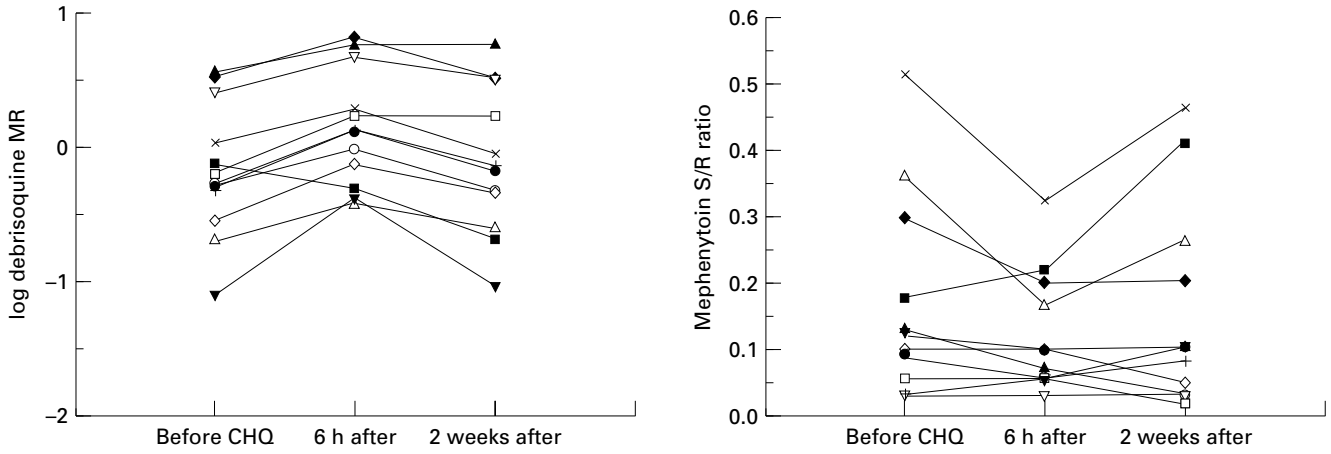

Figure 1 The effects of the loading dose of chloroquine (1500 mg) on the mephenytoin S/R ratios and the debrisoquine metabolic ratios (MR), 6 h and 2 weeks after the last dose of chloroquine in 12 Swedish Caucasian subjects. Each individual is indicated by a specific symbol.

Phenotyping and chloroquine treatment

Subjects were phenotyped for debrisoquine 4-hydroxylase and S-mephenytoin hydroxylase activities [9] 1 week before CHQ administration and after CHQ administration. Chloroquine was given as a single prophylactic dose (two tablets of 250 mg chloroquine phosphate, Pharmacia, Sweden) orally to the eleven Zimbabwean subjects 6 h before phenotyping commenced. Chloroquine was also given as a loading dose of 1500 mg chloroquine to the 12 white Swedish volunteers using the regimen typical for the first day of treatment of malaria (500 mg chloroquine phosphate (Klorokinfosfat, Pharmacia, Sweden) at 08.00 h, another 500 mg at 12.00 midday and finally 500 mg at 16.00 h). Phenotyping commenced 6 h later. The subjects were phenotyped again 2 weeks after the loading dose. Debrisoquine, 4-hydroxydebrisoquine and S- and R-mephenytoin concentrations were determined in urine for calculation of metabolic ratios [9–11].

Results

Figure 1 shows the individual metabolic ratio data for the effects of the loading dose of CHQ. Table 1 summarizes the effects of prophylactic and loading doses of CHQ on metabolic ratios of debrisoquine to 4-hydroxydebrisoquine and S-mephenytoin to R-mephenytoin. There were no statistically significant differences between the metabolic ratios before and after chloroquine treatment tested by either the Mann–Whitney test or the unpaired *t*-test. The changes, including negative changes, observed in metabolic ratios after chloroquine treatment may be a reflection of normal intraindividual variation of the ratios.

Discussion

Although CHQ has been found to be an effective inhibitor *in vitro* of CYP2D6 catalysed bufuralol

Table 1 The effect of chloroquine on metabolic ratios for debrisoquine 4-hydroxylase and S-mephenytoin hydroxylase

	Metabolic ratios			
	Debrisoquine		*S-Mephenytoin*	
CHQ treatment	*Mean ± s.d.* range	**(95% CI)* (P *value)*	*Mean ± s.d. range*	**(95% CI)* (P *value)*
Prophylactic dose				
Before CHQ ($n=11$)	3.13 ± 3.27		0.35 ± 0.36	
	0.56–10.7		0.06–1.07	
6 h after CHQ ($n=11$)	3.38 ± 3.59	(−2.81 to 3.30)	0.25 ± 0.27	(−0.38 to 0.18)
	0.66–12.0	($P=0.87$)	0.03–0.83	($P=0.46$)
Loading dose				
Before CHQ ($n=12$)	1.10 ± 1.15		0.17 ± 0.15	
	0.08–3.43		0.03–0.51	
6 h after CHQ ($n=12$)	2.05 ± 2.03	(−0.47 to 2.37)	0.12 ± 0.08	(−0.16 to 0.05)
	0.37–5.68	($P=0.18$)	0.03–0.32	($P=0.31$)
2 weeks after CHQ ($n=12$)	1.43 ± 1.68	(−0.89 to 1.55)	0.15 ± 0.15	(−0.14 to 0.11)
	0.09–5.55	($P=0.58$)	0.02–0.46	($P=0.80$)

* Unpaired *t*-test: 95% confidence interval (CI) for the difference between the means before and after chloroquine treatment and corresponding two-tailed *P* value.

1′-hydroxylation ($K_i = 12$ μM) [7] and metoprolol α-hydroxylation ($K_i = 0.18$ μM) [6], our study shows that CHQ did not alter significantly CYP2D6 activity *in vivo*. This latter observation is surprising especially as chloroquine concentrations in the liver must have been in excess of these K_i values [12]. This highlights the fact that *in vitro* findings cannot always be extrapolated to the *in vivo* situation. A possible explanation is that CHQ is available to the enzyme active site *in vitro*, but the drug might be distributed *in vivo* such that it is not available for interaction with CYP2D6. The observation that chloroquine has no statistically significant effect on the activities of debrisoquine hydroxylase or S-mephenytoin hydroxylase suggests that chloroquine is not likely to interfere with the metabolism *in vivo* of other drugs which are substrates of CYP2D6 or CYP2C19. The suggestion, therefore, that residual chloroquine could be one of the causes of inconsistent phenotyping results is probably not correct. Concerning possible effects of a loading dose of chloroquine, it is highly unlikely that anyone in the first stages of treatment for malaria (i.e. 6 h after a loading dose of CHQ) has participated in a phenotyping study. However, caution must be exercised when extrapolating data to a clinical situation involving long term prophylaxis.

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References

1 Kalow W, Bertilsson L. Interethnic factors affecting drug response. *Adv Drug Rev* 1994; **25**: 1–53.

2 Lennard MS, Iyun AO, Jackson PR, Tucker GT, Woods HF. Evidence for dissociation in the control of sparteine, debrisoquine and metoprolol metabolism in Nigerians. *Pharmacogenetics* 1992; **2**: 89–92.

3 Iyun AO, Tucker GT, Woods HF, Lennard MS. The 4-hydroxylation of S-mephenytoin in Nigerians: A population study. *Microsomes and drug oxidations—VIIIth International Symposium*, eds Ingelman-Sundberg, M, Gustafsson J-A, Orrenius S. Stockholm. Abstr **373**.

4 Masimirembwa CM, Bertilsson L, Hasler JA, Johansson I, Ingelman-Sundberg M. Genotyping and phenotyping of mephenytoin hydroxylase (CYP2C19) in the Shona population of Zimbabwe. *Clin Pharmacol Ther* 1995; **57**: 651–61.

5 Brosen K, Gram LF, Haghfelt T, Bertilsson L. Extensive metabolisers of debrisoquine become poor metabolisers during quinidine treatment. *Pharmacol Toxicol* 1987; **60**: 312–314.

6 Lancaster DL, Adio RA, Tai KK, *et al.* Inhibition of metoprolol metabolism by chloroquine and other antimalarial drugs. *J Pharm Pharmacol* 1990; **42**: 267–271.

7 Masimirembwa CM, Hasler JA, Johansson I. Inhibitory effects of antiparasitic drugs on cytochrome P4502D6. *Eur J Clin Pharmacol* 1995; **48**: 35–38.

8 Alvan G, Ekman L, Lindström B. Determination of chloroquine and its desethyl metabolite in plasma, red blood cells and urine by liquid chromatography. *J Chromatogr* 1982; **229**: 241–247.

9 Sanz EJ, Villen T, Alm C, Bertilsson L. S-mephenytoin hydroxylation phenotype in a Swedish population determined after coadministration with debrisoquin. *Clin Pharmacol Ther* 1989; **45**: 495–499.

10 Lennard MS, Silas JH, Smith AJ, Tucker GT. Determination of debrisoquine and its 4-hydroxymetabolite in biological fluids by gas chromatography with flame-ionization and nitrogen-selective detection. *J Chromatogr* 1977; **133**: 161–166.

11 Wedlund PJ, Sweetman BJ, McAllister CB, Branch RA, Wilkinson GR. Direct enantiomer resolution of mephenytoin and its N-demethylated metabolite in plasma and blood using chiral capillary gas chromatigraphy. *J Chromatogr* 1984; **307**: 121–127.

12 Prouty RW, Kuroda K. Spectrophotometric determination and distribution of chloroquine in human tissues. *J Lab Clin Med* 1958; **52**: 477–480.

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