Chloroquine modulation of specific metabolizing enzymes activities: investigation with selective five drug cocktail

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Aims The aim of this study was to investigate whether chloroquine can inhibit drug metabolism in humans, if such inhibition is general or selective for certain enzymes and evaluate the potential for and clinical significance of any drug-drug interactions when chloroquine is co-administered with other drugs.

Methods The study was conducted in fourteen normal non-smoking healthy male volunteers using a cocktail of five drugs consisting of caffeine, mephenytoin, debrisoquine, chlorzoxazone and dapsone to assess activities of cytochromes P450 (CYP) 1A2, 2C19, 2D6, 2E1 and 3A4 respectively. Dapsone was also used to assess *N*-acetyltransferase activity. The activities were assessed at baseline, after one and seven daily doses (250 mg daily) of chloroquine and 7 and 14 days after stopping chloroquine dosing.

Results Chloroquine caused a progressive and significant decrease in CYP2D6 activity as measured by debrisoquine metabolism from first to seventh dose and the activity returned to baseline gradually over 14 days after stopping administration. There was no effect on the metabolism of any of the other probe drugs.

Conclusions Chloroquine has been shown to be capable of inhibiting the activity of CYP2D6 *in vivo* in humans. This effect is selective as activities of other enzymes investigated were not affected. The effect was modest but suggests a potential for drug-drug interactions when co-administered with other drugs that are substrates for this enzyme. The clinical significance of such an interaction will depend on the therapeutic index of any drug involved.

Keywords: chloroquine, metabolism, inhibition, drug cocktail, selective inhibition

Introduction

Chloroquine is a 4-aminoquinoline derivative that is the most widely used drug for the prevention and treatment of malaria [1–3]. It is also indicated in other conditions such as autoimmune diseases including rheumatoid arthritis and systemic lupus erythematosus [4–6]; and diabetes [7, 8]. Apart from these therapeutic indications, chloroquine has been shown to have other effects that may be important in its usage because of their potential to modulate drug metabolism and cause drug-drug interactions since it is generally used in combination with other drugs. These include, inhibition of drug metabolism [9–11], reduction in co-factors that may be important for metabolism [12] and inhibition of protein synthesis [13, 14]. Such effects have not been demonstrated unequivocally; it does not alter the metabolism of some compounds [15, 16]. Consequently, the effect of chloroquine on metabolizing activities is not well understood and the reason(s) for these conflicting results is unclear. More specifically, chloroquine has been shown to be a very potent inhibitor of CYP2D in rats [9] but the effect in humans and on other enzymes which may be affected have not been fully elucidated. It is, therefore, difficult to anticipate the extent of drug-drug interactions that may occur with chloroquine.

In an attempt to investigate this effect, we have employed a cocktail approach to assess the effect of chloroquine on activities of multiple enzymes simultaneously. The approach involved the use of a combination of five drugs that have been shown to be substrates for certain specific metabolizing enzymes. These include caffeine (CYP1A2 [17]), mephenytoin (CYP2C19 [18]), debrisoquine (CYP2D6 [19]), chlorzoxazone (CYP2E1 [20]), and dapsone (CYP3A4 [21]). In addition, dapsone was used to assess the activity of *N*-acetyltransferase [22]. The objective of the study was to characterize the *in vivo* inhibition of CYP2D6 activity by chloroquine in humans. Other enzymes will serve as negative controls if the effect is selective. This cocktail has previously been validated and it was shown that the ability of these five drugs to measure the activity of their respective enzymes is not affected by their co-administration [23]. The study investigated the effect of a single and multiple daily doses of chloroquine on the activities of these enzymes as well as recovery from any such effects after the discontinuation of chloroquine.

Methods

The study was conducted in fourteen non-smoking healthy male volunteer subjects aged between 19 and 32 years

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(23.4 ± 3.7, mean ± s.d.) and weighing between 64 and 88 kg (72.7 ± 7.2, mean ± s.d.). The number of subjects used was based on power analysis calculations of the number of subjects that would be required to demonstrate a 20% change or greater in the activities of these enzymes from baseline at $P<0.05$ with 80% power. The study was approved by the University of Pittsburgh Institutional Review Board for Biomedical Research and was conducted after obtaining written informed consent from each subject. No subject was a poor metabolizer of any of the CYP polymorphic enzymes based on phenotypic determinations. The health status of the subjects was assessed by medical history, physical examination and routine laboratory tests to assess hepatic, renal and cardiac functions. Subjects were required to abstain from taking any medication or alcoholic beverage 1 week prior to and during the period of the study and also from any caffeine containing products from 48 h before each study day.

The study was conducted at the General Clinical Research Center where subjects were admitted on the morning of the study day. Subjects fasted from 24.00 h midnight the night before the study and until 2 h after the administration of the drugs. The study was conducted by the simultaneous oral administration of low doses of the five drugs: caffeine (100 mg), racemic mephenytoin (100 mg), debrisoquine (10 mg), chlorzoxazone (250 mg) and dapsone (100 mg). Blood samples (30 ml) were obtained before and at 4 and 8 h after dosage into tubes containing EDTA as anticoagulant. Plasma was obtained by centrifugation at 9000 *g* for 10 min at 4° C. Total voided urine was collected for 8 h after administration of the cocktail drugs into containers with 1 g ascorbic acid as preservative for the unstable dapsone hydroxylamine metabolite. Total volume of urine was recorded and an aliquot, together with the plasma samples, were stored frozen at −20° C until analysis.

Each subject was studied on five different occasions. The baseline cocktail study was conducted before administration of chloroquine. This was separated by at least 2 weeks from the chloroquine intervention phase of the study. The cocktail study was repeated after a single oral dose (250 mg) of chloroquine phosphate as described above. The cocktail drugs were given 2 h after the dose of chloroquine. Chloroquine administration was continued on a daily dosing regimen of 250 mg for another 6 days. After the seventh daily dose, the cocktail study was repeated. To assess recovery from any effect of chloroquine, the cocktail study was repeated at 7 and 14 days after discontinuation of chloroquine.

Drugs and metabolites were determined in these samples and the respective phenotypic measures for the enzymes were calculated as previously described [23, 24]: paraxanthine to caffeine ratio in 8 h plasma sample (CYP1A2), 4′-hydroxymephenytoin recovery and *S*/*R* enantiomeric ratio in 8 h urine (CYP2C19), debrisoquine recovery ratio, DBRR (CYP2D6), 6-hydroxychlorzoxazone to chlorzoxazone ratio in 4 h plasma sample (CYP2E1), dapsone recovery ratio, DPRR (CYP3A4) and monoacetyldapsone to dapsone ratio in 8 h plasma sample (*N*-acetyltransferase). The measures on the different occasions were compared by repeated measures ANOVA and the Student-Newman-Keuls multiple comparisons test. The level of significance was set at $P<0.05$.

Results

One of the subjects unavoidably missed one of the legs of the study and was studied on four out of the five occasions required. The leg missed was the 7 days post chloroquine administration study. As a result, this subject could not be included in the statistical analysis by repeated measures ANOVA or the Student-Newman-Keuls multiple comparisons test because of missing data. However, his exclusion did not alter the mean of the group as shown for example by the debrisoquine recovery ratio. For the five legs with the subject included, the means and standard deviations were: 0.56 ± 0.15, 0.52 ± 0.13, 0.45 ± 0.11, 0.52 ± 0.13 and 0.56 ± 0.13 and the corresponding numbers with the subject excluded were: 0.56 ± 0.15, 0.52 ± 0.13, 0.46 ± 0.12, 0.52 ± 0.13 and 0.55 ± 0.13.

Chloroquine produced a reduction in the metabolism of debrisoquine as evaluated by the debrisoquine recovery ratio (DBRR), a measure of CYP2D6 activity. This reduction was progressive from the first to the seventh dose (Figure 1 and Table 1). This decrease in metabolism was modest (about 7% after the first dose and about 18% after seven doses) but statistically significant by repeated measures ANOVA ($P<0.0001$). This reduction in metabolism was reversed and the activity returned to baseline level gradually within 2 weeks of stopping chloroquine (Figure 1 and Table 1). Multiple comparisons by Student-Newman-Keuls test showed that DBRR values after seven daily doses of chloroquine were significantly ($P<0.05$) different from baseline values as well as values after one dose of chloroquine and after 7 and 14 days of stopping chloroquine. Also, there was a relationship between the baseline DBRR measure and the change in DBRR after one dose of chloroquine ($r=-0.52$, $P=0.05$), after seven daily doses of chloroquine ($r=-0.63$, $P=0.01$) and 7 days after stopping chloroquine ($r=-0.59$, $P=0.03$) or with all of them combined ($r=-0.53$, $P=0.0004$).

There was no change in any of the other phenotypic measures as determined by caffeine, mephenytoin, chlorzox-

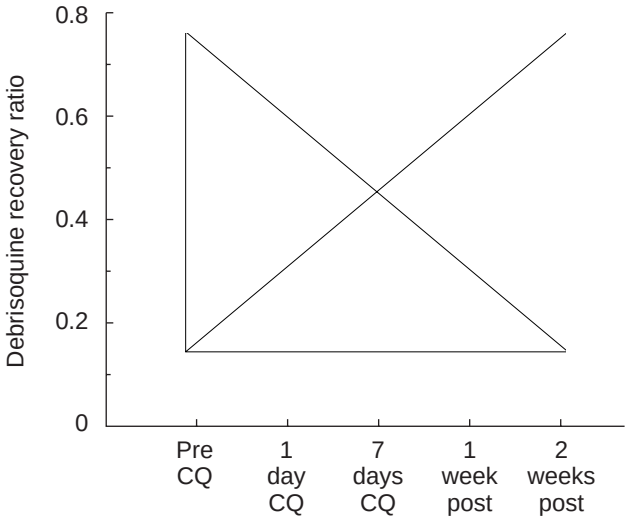

Figure 1 Debrisoquine recovery ratio before, after one and seven daily doses of chloroquine and 7 and 14 days after stopping chloroquine dosing in 14 young healthy subjects. Significantly different ($P<0.0001$) by repeated measures ANOVA.

Table 1 Phenotypic measures of specific metabolizing enzymes at baseline, during multiple daily chloroquine dosing and during washout after chloroquine dosing (mean (s.d.)).

Enzyme/ measure	*Baseline*	*One dose*	*Seven doses*	*7 days post*	*14 days post*
CYP1A2	0.64	0.62	0.67	0.55	0.64
	(0.24)	(0.25)	(0.22)	(0.25)	(0.33)
CYP2C19					
4′-OHMEP (μmol)	128.6	133.1	136.3	134.9	137.6
	(40.5)	(40.1)	(29.9)	(36.2)	(23.4)
S/R ratio	0.18	0.15	0.14	0.13	0.15
	(0.15)	(0.14)	(0.10)	(0.08)	(0.13)
CYP2D6	0.56	0.52	0.45	0.52	0.56
	(0.15)	(0.13)	(0.11)	(0.13)	(0.13)
CYP2E1	0.64	0.63	0.57	0.56	0.67
	(0.22)	(0.21)	(0.22)	(0.25)	(0.26)
CYP3A4	0.45	0.45	0.48	0.45	0.46
	(0.16)	(0.15)	(0.12)	(0.15)	(0.14)
NAT	0.30	0.29	0.29	0.31	0.29
	(0.27)	(0.25)	(0.26)	(0.27)	(0.24)

azone and dapsone as a result of chloroquine administration (Table 1). The lack of effect on *N*-acetyltransferase was evident even when subjects were divided into fast and slow acetylators (result not shown).

Discussion

This study has shown that chloroquine can inhibit the activity of metabolizing enzymes and that this effect is selective, affecting some enzymes while not affecting others. Specifically, it has been shown that chloroquine inhibited the activity of CYP2D6 as measured by the debrisoquine recovery ratio. Activities of CYP1A2, CYP2C19, CYP2E1 and CYP3A4 as measured by caffeine, mephenytoin, chlorzoxazone and dapsone respectively and *N*-acetyltransferase as measured by dapsone were not affected.

The effect on CYP2D6 appears to be concentration dependent as it gradually progressed from a 7% decrease after a single dose to about 18% decrease after seven doses of chloroquine. Given a reported half-life of up to 40 days for chloroquine [3], it would be expected that it would accumulate in the body during the 7 day dosing period. Thus, it can be inferred that the increasing inhibitory effect was due to increasing chloroquine concentration in the body though, chloroquine concentrations were not measured in this study. The observation of a gradual return to baseline over 2 weeks after stopping chloroquine administration is consistent with this inference and reported long half life of chloroquine, as it can be interpreted as being due to a progressive decline in chloroquine concentration.

The observed effect on CYP2D6 by chloroquine in this study is in agreement with a previous study [9]. Chloroquine was a potent inhibitor of metoprolol metabolism mediated by CYP2D in rat and human liver microsomes though in human microsomes the drug was two orders of magnitude less potent as an inhibitor. Also, chloroquine, at doses comparable with humans on a per weight basis, inhibited this activity in anesthetized rats. By contrast, the conclusions of our study, with respect to CYP2D6 activity, are different from those of a recent report by Masimirembwa *et al.* [15]. Also using debrisoquine, but with activity measured as the metabolic ratio (debrisoquine/4-hydroxydebrisoquine in urine), they reported that neither a single dose (2×250 mg tablets) nor a loading dose (1500 mg day^{-1} in three divided equal doses) of chloroquine caused a statistically significant change in CYP2D6 activity. A cursory look at the conclusions of their study and ours will suggest that they are diametrically opposed in their findings and conclusions. However, a re-examination of the results in that report will reveal that there was a trend towards a decrease in CYP2D6 activity as measured by the debrisoquine metabolic ratio with 11 of 12 subjects showing increases in this measure. The difference did not reach statistical significance in spite of a doubling of the metabolic ratio after the loading dose study. This is the same trend observed in our study but here it did reach statistical significance. The difference between the two studies could be due to the choice of statistical test used in that study. The results were compared by unpaired *t*-test in a case where paired *t*-test or repeated measures ANOVA, as used in this study, might have been more appropriate. The progressive decline in the measure with repeated dosing of chloroquine and progressive recovery on stopping the administration of chloroquine in our study strongly suggest that chloroquine was inhibiting CYP2D6 activity. A return towards baseline activity was also observed in the study by Masimirembwa *et al.* [15].

This apparent inhibitory effect of chloroquine on CYP2D6 is consistent with previous reports about the ability of chloroquine to inhibit metabolizing activities [9–11]. These previous reports were based on *in vitro* and animal studies and this study has shown that similar effects may be obtained *in vivo* in humans.

In contrast to the effect on CYP2D6, chloroquine did not affect the activities of CYP1A2, CYP2C19, CYP2E1, CYP3A4 or *N*-acetyltransferase. This result is in agreement with Masimirembwa *et al.* [15] who did not see any effect of chloroquine on *S*-mephenytoin hydroxylation by CYP2C19. The lack of effect on these enzyme activities provides a good negative contrast and some validation for the positive inhibitory effect on CYP2D6 and clearly

illustrate the selective nature of the effect. This may explain why some other studies have found no effect of chloroquine on certain metabolizing activities [15, 16] and illustrates the importance of evaluating multiple enzymes before any general conclusions can be drawn on the modulating potential of any agent.

In summary, this study has shown that chloroquine can selectively inhibit the activity of CYP2D6 while not affecting CYP1A2, CYP2C19, CYP2E1, CYP3A4 or *N*-acetyltransferase in humans. It has extended previous findings because of the number of specific enzymes evaluated. The study has demonstrated a potential for drug-drug interaction whenever chloroquine and substrates of CYP2D6 are co-administered *in vivo*. Given its very long half-life (about 40 days) this potential may still be relevant for a long time after stopping administration in patients who have undergone prolonged treatment with high dose chloroquine. While the effects shown here are modest, a clinically significant interaction may occur with drugs that have narrow therapeutic indices or that are activated by the affected enzyme such as codeine and hydrocodone [25–28]. This study has also shown the utility of the cocktail approach used in this study for assessing differential regulation and modulation of the enzymes being assessed. Given that the enzymes evaluated by this cocktail are involved in the metabolism of greater than 90% of drugs in clinical use, it has potentially wide application in predicting the effect of a compound on specific metabolizing enzymes.

Sincere gratitude is expressed to Roche Pharmaceuticals for the supply of debrisoquine and 4-hydroxydebrisoquine reference standards, Dr G. R. Wilkinson of Vanderbilt University, Nashville, TN for the gift of 4′-hydroxy-mephenytoin standard and McNeil Pharmaceuticals of Raritan, New Jersey, for the gift of 6-hydroxychlorzoxazone. The technical assistance of James Porter and Cheryl Galloway is gratefully acknowledged.

The study was supported in part by Public Health Service grants GM49356 and NIH/NCRR/GCRC #5M01 RR00056 and the Central Research Development Fund of the University of Pittsburgh.

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(*Received 30 September 1997, accepted 17 March 1998*)