Quinine Compared to 4β-Hydroxycholesterol and Midazolam as Markers for CYP3A Induction by Rifampicin

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Summary: When developing new drugs appropriate markers for detecting induction and inhibition of cytochrome P450 3A enzymes (CYP3A) are needed. The aim of the present study was to evaluate the quinine/3-hydroxyquinine metabolic ratio (quinine MR) with other suggested markers for CYP3A induction: endogenously formed 4β-hydroxycholesterol, midazolam clearance in plasma and the 6β-hydroxycortisol/cortisol ratio in urine. We have previously performed a clinical trial in which 24 healthy subjects were randomized to take 10, 20 or 100 mg daily doses of rifampicin for 14 days (n = 8 in each group) to achieve a low and moderate CYP3A induction. In newly analyzed data from this study we can show that the quinine MR could detect CYP3A-induction even at the lowest dose of rifampicin (10 mg) (p < 0.01), comparable to a 4β-hydroxycholesterol/cortisol ratio and midazolam clearance. The median fold-induction for the quinine MR compared to baseline was 1.7, 1.8 and 2.6 for the three dosing groups (10, 20 and 100 mg). In conclusion, in this study the quinine MR was comparable to midazolam clearance as a measure of CYP3A activity but easier to determine since only a single blood sample needs to be drawn.

Keywords: cytochrome P450 3A4; quinine; cortisol ratio; midazolam clearance; 4β-hydroxycholesterol; drug development

Introduction

Cytochrome P450 3A enzymes (CYP3A) are considered to be the most important enzymes catalyzing drug metabolism with broad substrate specificity. CYP3A enzymes may be involved in drug-drug interactions both as a result of drug inhibition and induction.¹–³ When new drugs are developed, appropriate markers for detecting induction and inhibition of CYP3A are needed to predict drug-drug interactions. We recently performed a clinical trial to evaluate different markers for CYP3A activity.⁴ In this trial 24 healthy subjects were randomized to take 10, 20 or 100 mg daily doses of rifampicin for 14 days (n = 8 in each group) to achieve a low and moderate CYP3A induction. The aim of the study was to evaluate endogenously formed 4β-hydroxycholesterol with other suggested markers for CYP3A activity, including midazolam clearance in plasma and the 6β-hydroxycortisol/cortisol ratio in urine. The design of the study is described in details in our previous article.⁴ From this study we have now analyzed the quinine/
3-hydroxyquinine metabolic ratio (quinine MR) in plasma, another suggested marker for CYP3A activity.

The aim of the present study was to evaluate the quinine MR with other suggested markers for CYP3A induction: endogenously formed 4β-hydroxycholesterol, midazolam clearance in plasma and the 6β-hydroxycortisol/cortisol ratio in urine.

Materials and Methods

Study design: In an open randomized controlled study, 24 Swedish Caucasian healthy volunteers were randomized to take 10, 20 or 100 mg rifampicin per day for 2 weeks to achieve CYP3A induction. There were 8 subjects in each treatment group. Determinations of the midazolam AUC, 4β-hydroxycholesterol ratio, quinine MR and 6β-hydroxycortisol ratio were performed at baseline and after 2 weeks of medication. In addition the 4β-hydroxycholesterol ratio, quinine MR and 6β-hydroxycortisol ratio were determined 2 weeks after termination of medication (4 weeks after study start) but the midazolam AUC was not determined at this time point. The quinine tablet (250 mg quinine hydrochloride, Recip AB, Solna, Sweden) was taken by the volunteers at 18:00 in the evening and the blood sample was drawn 8:00 the next morning, i.e. 14 h after the dose. The study was performed at Clinical Pharmacology Trial Unit, Karolinska University Hospital Huddinge during April–June 2011. The study was approved by the local Ethics Committee (Dnr: 2010/1734-31/1) and the Swedish Medical Product Agency and was performed in accordance with the declaration of Helsinki. Written informed consent was obtained from all study participants. The EudraCT number is 2010-023014-31. The safety of the volunteers was guaranteed during the study period by safety screening for hematological, liver and kidney status (clinical chemistry analysis) at baseline, day 15 and day 28. In addition, a physical examination was carried out by the study physician at the same time points. No serious adverse events were reported during the study period.

The study design and measurements of 4β-hydroxycholesterol, cholesterol, 6β-hydroxycortisol, cortisol and midazolam are described in detail in our previous study.4)

Measurements of quinine and 3-hydroxyquinine: The concentration of quinine and its metabolite (3S)-3-hydroxy quinine were determined by liquid chromatography tandem mass spectrometry (LC-MS/MS) following sample preparation by protein precipitation. An aliquot of 200 µL internal standard solution (0.1% formic acid in acetonitrile containing 0.2 µM internal standards quinine-d3 and (3S)-3-hydroxy quinine-vinyl-d3) was added to 100 µL of sample. After vortexing and centrifugation, 2 µL of the extract was injected into the LC-MS/MS system. Separation of the analytes was achieved on an Acquity UPLC BEH C18-column (2.1 x 50 mm 1.7 μm), using a gradient run with mobile phase A (11 mM ammonium formate) and mobile phase B (0.1% formic acid in acetonitrile). The analytes were detected using a Micromass Quattro Premier XE mass spectrometer operating in positive electrospray ionization (ESI) mode utilizing selected reaction monitoring (SRM) for the transitions 325 → 160 m/z for quinine, 341 → 160 m/z for (3S)-3-hydroxy quinine, 328 → 163 m/z for quinine-d3 and 344 → 160 m/z for (3S)-3-hydroxy quinine-vinyl-d3.

Statistical methods: Statistical analyses were performed using GraphPad Prism software version 5.03 (San Diego, CA). The fold-induction among the three dosing-groups was analyzed by a one-way Kruskal-Wallis test (Fig. 1) followed by an un-paired, two-tailed Mann-Whitney test between the different groups. In the statistical analysis of comparison between the quinine MR and 4β-hydroxycholesterol ratio the Wilcoxon matched-pairs signed rank test was used (Fig. 2).

Results

In a fold-induction analysis the CYP3A-induction after 2 weeks of daily 10, 20 and 100 mg rifampicin treatment could be detected by the quinine MR (p < 0.01) even at the lowest dose of rifampicin (10 mg) as shown in Figure 1. The median fold-induction from baseline was 1.7, 1.8 and 2.6 for the three dosing groups (10, 20 and 100 mg). Statistical analysis comparing the 2 weeks’ values with the baseline values in the different dosing-groups showed that the quinine MR was comparable to the 4β-hydroxycholesterol ratio and estimated midazolam clearance for detecting induction (p < 0.01). All three markers were superior to the 6β-hydroxycortisol ratio, which showed more divergent results in CYP3A induction between the different volunteers (p < 0.05).

A Mann-Whitney test between the different dosing groups showed a statistical significant difference in the quinine MR between 100 and 20 mg and 100 and 10 mg rifampicin (both p < 0.001) but not between 10 mg and 20 mg.

In Figure 2 the values of the quinine MR and 4β-hydroxycholesterol ratio can be followed through the study period from baseline, after 2 weeks of rifampicin treatment and 2 weeks after termination of rifampicin treatment (4 weeks after study start). Midazolam clearance was not determined after 4 weeks, because of ethical problems over performing the midazolam test a third time in these subjects. Both the quinine MR and 4β-hydroxycholesterol ratio could detect the decrease in CYP3A activity after the termination of rifampicin treatment, week 4 compared with week 2. Four weeks after study start the quinine MR had returned to baseline values (Fig. 2). In contrast, the 6β-hydroxycortisol ratio still showed significantly higher values 4 weeks after study start compared to baseline in all three dosing groups, due to the long half-life of this compound (Fig. 2).
Discussion

In our previous report we showed that CYP3A induction could be detected, even at the lowest dose of rifampicin (10 mg), by the estimated midazolam clearance and 4β-hydroxycholesterol/cholesterol ratio (both \( p < 0.01 \)) and by the 6β-hydroxycortisol ratio (\( p < 0.05 \)). Here we show that the quinine MR can also detect CYP3A-induction at the lowest dose of rifampicin (10 mg) as depicted in Figure 1.

The advantage of the quinine MR compared to midazolam is that the probe drug (quinine 250 mg) could be taken by the volunteer in the afternoon/evening at home and only a single blood sample needs to be drawn the next morning in the hospital. When using midazolam as a probe drug 8–10 blood samples need to be drawn during 8–10 h after the administration and the volunteer has to stay at the department during this time. There are contradictory reports about whether midazolam clearance can be determined from a single blood sample or not. Long-term treatment with quinine is sometimes associated with rare side effects such as hematological toxicity and nephrotoxicity. More common side effects are nausea, headache or dizziness. However, when using quinine as a probe drug only a single dose is given and a relative low dose is used, 250 mg, to be compared with doses during therapeutic use that could be 600 mg × 3 daily. We believe that quinine is a comparatively safe drug to give at this low dose and in the present study no adverse reactions possibly associated with quinine were reported. We have previously given quinine as a probe drug to several hundreds of healthy subjects of different origin without any serious events.

A major advantage with the endogenously formed 4β-hydroxycholesterol as a CYP3A marker is that no probe drug needs to be administrated and only one blood sample, at any time, is required. However, a disadvantage of 4β-hydroxycholesterol is the long half-life of this compound (17 days). Thus, 4β-hydroxycholesterol is not optimal for detecting CYP3A inhibition, and to study CYP3A induction the study period should be at least 2 weeks. Midazolam as well as the quinine MR is superior to 4β-hydroxycholesterol in detecting inhibition of CYP3A4. In line with this, the present study shows that the CYP3A activity was back to baseline values when measured with the quinine MR but not when measured with 4β-hydroxycholesterol 2 weeks after 100 mg rifampicin treatment was terminated (4 weeks after study start) (Fig. 2). Midazolam clearance was not measured at this time point (4 weeks after study start) but most probably would have shown the same results as for the quinine MR.

Besides the previously described endogenous markers for CYP3A activity, 4β-hydroxycholesterol in serum and the 6β-hydroxycortisol ratio in urine studied here, new interesting endogenous markers for CYP3A-activity have been suggested by Shin and co-workers. In this recently performed study it was shown that metabolomic analysis of urine samples could be used to predict midazolam clearance.

We suggest that the different CYP3A markers should be further studied to understand their merits and limitations. As an example, in addition to measuring the CYP3A4-activity, the 4β-hydroxycholesterol ratio, 6β-hydroxycortisol ratio and quinine MR are also affected by CYP3A5 genotype while midazolam clearance is not. Midazolam is extensively metabolised in the intestine; thus midazolam also measures intestinal CYP3A activity while we assume that the 4β-hydroxycholesterol ratio and 6β-hydroxycortisol ratio do not measure intestinal CYP3A-activity to the same extent.

Fig. 2. Comparison of two different markers for CYP3A activity: quinine metabolic ratio (quinine MR) and 4β-hydroxycholesterol ratio (4β-OHchol R) at baseline and after CYP3A induction achieved by rifampicin treatment for 2 weeks (10, 20 or 100 mg daily) and 2 weeks after rifampicin administration was terminated (4 weeks) statistically significant differences were calculated using the Wilcoxon matched pairs single rank test.

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As the quinine MR is much easier to determine than midazolam clearance, we suggest that a combination of 4β-hydroxycholesterol and the quinine MR could be used as a simple and accurate measure of both CYP3A induction and inhibition. Quinine might be administrated by the subjects at home in the afternoon. Next morning a single blood sample might be drawn and used for both 4β-hydroxycholesterol and quinine analysis. Compared to midazolam clearance determination this is a much easier procedure.

In conclusion, the present study suggests the quinine MR is comparable to midazolam and 4β-hydroxycholesterol for determining CYP3A induction.

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References


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