Cytochrome P450 3A (CYP3A) enzymes are involved in the metabolism of numerous drugs as well as endogenous compounds such as steroid substrates [1,2]. The enzyme activity is highly variable between individuals and may be strongly induced or inhibited by several drugs and foods. It is also affected by CYP3A5 genotype [3]. It is therefore important to be able to monitor the enzyme activity in a simple way, both in patients and during development of new drugs. At present, the most common method to monitor the enzyme activity is to use probe drugs such as midazolam [4] or quinine [5], but endogenous markers such as 6-hydroxycortisol have also been used [6,7]. We have recently shown that the endogenous oxysterol 4β-hydroxycholesterol may be used as a marker for the drug-metabolizing enzymes cytochrome P450 3A (CYP3A). The primary aim of this study was to investigate the effect of statin treatment on plasma 4β-hydroxycholesterol concentrations. Plasma samples from a previously performed clinical study where gallstone patients had been treated with placebo (n = 6), 20 mg fluvastatin (n = 9) or 80 mg atorvastatin (n = 9) daily for 4 weeks were analysed. Hepatic CYP3A mRNA levels had previously been shown to be unchanged in all three treatment groups. Plasma 4β-hydroxycholesterol did not change significantly (p = 0.92) in the placebo group, but treatment with low-dose fluvastatin or high-dose atorvastatin resulted in reductions in plasma concentration of 10.7% (p < 0.05) and 36.5% (p < 0.01), respectively. However, the 4β-hydroxycholesterol/cholesterol ratio did not change significantly for the patients receiving placebo or patients receiving low-dose fluvastatin. The ratio for patients receiving high-dose atorvastatin increased by 12% (p < 0.05). In conclusion, the total plasma cholesterol level is an important determinant for the plasma 4β-hydroxycholesterol level.

Materials and Methods

Patients and treatments. This study took advantage of an earlier study in which 37 normocholesterolaemic gallstone patients were randomized to treatment with placebo, 20 mg/day fluvastatin or 80 mg/day atorvastatin for 4 weeks in order to achieve different levels of inhibition of cholesterol synthesis [14]. Written informed consent was obtained from all patients before inclusion into the study, which was approved by the Human Ethics Committee of Karolinska Institutet (Dnr 9-287, approval date 1999-10-18) and by the Swedish Medical Products Agency and was carried out in accordance with the Declaration of Helsinki. Samples from 24 patients from this study were still available and analysed for 4β-hydroxycholesterol. From 14 patients of the original
study, there was unfortunately no plasma left. Among the 24 patients included in this study, there were eight men, seven post-menopausal women and nine fertile women. The mean age was 50 ± 4 years.

**Determination of 4β-hydroxycholesterol.** The oxysterol 4β-hydroxycholesterol was measured as described previously [15]. Briefly, isotope dilution gas chromatography–mass spectrometry was used and deuterium labelled 4β-hydroxycholesterol was used as internal standard. The within-day variation was 4.5% and the between-day variation was 8.2% at 25 ng/mL. The method was linear up to 600 ng/mL.

**Statistics.** All statistical tests were performed using GraphPad Prism v. 6.00 (GraphPad Software, Inc., La Jolla, CA, USA), and values of p < 0.05 were considered statistically significant. Comparisons before and after treatment were carried out using the nonparametric Wilcoxon-matched pairs signed rank test.

**Results**

As shown in the original study, the mean plasma total cholesterol concentration in the placebo group changed from 5.38 to 5.30 mmol/L (−1.5%, not significant) while low-dose fluvastatin treatment reduced cholesterol by 18% (p < 0.05) and high-dose atorvastatin treatment resulted in a 44% decrease (p < 0.001) [14]. Hepatic mRNA levels of CYP3A4 have previously been analysed in all individuals in this cohort and showed no significant differences between the three groups [16].

The plasma concentration of 4β-hydroxycholesterol did not change significantly (p = 0.92) in the placebo group as shown in the upper row of fig. 1. However, treatment with low-dose fluvastatin or high-dose atorvastatin resulted in reductions in plasma concentration of 10.7% (p < 0.05) and 36.5% (p < 0.01), respectively (fig. 1, upper row).

The 4β-hydroxycholesterol/cholesterol ratio for the three groups are shown in the lower row of fig. 1. The ratio did not change significantly for the patients receiving placebo or patients receiving low-dose fluvastatin. The ratio for patients receiving high-dose atorvastatin increased by 12% (p < 0.05).

**Discussion**

Oxysterols are transported together with cholesterol in lipoproteins in the circulation [17]. Therefore, changes in plasma cholesterol concentrations may affect plasma oxysterol concentrations. We have shown earlier that the distribution of 4β-hydroxycholesterol in different lipoprotein classes is very similar to the distribution for cholesterol [15]. The major part of 4β-hydroxycholesterol is found in the LDL fraction [15]. Statin treatment resulted in reduced plasma cholesterol as well as reduced plasma apo B [14]. The result is a reduction in plasma lipoproteins which will affect the transport of lipoprotein-bound lipids.

Fig. 1. 4β-hydroxycholesterol (4b-OHchol) and the ratio 4β-hydroxycholesterol/cholesterol (4b-OHchol/chol ratio) in plasma before and after 4 weeks of placebo (n = 6), fluvastatin (n = 9) or atorvastatin (n = 9) treatment. Statistical analysis was carried out using Wilcoxon-matched pairs signed rank test, ns = not significant.
Statin treatment did not affect 7α-hydroxy-4-cholesten-3-one/cholesterol, a marker of bile acid synthesis [14]. 7α-Hydroxy-4-cholesten-3-one is formed from cholesterol by two enzymatic steps. It has earlier been shown by Honda et al. [18] that the ratio of 7α-hydroxy-4-cholesten-3-one to cholesterol is a better marker for hepatic bile acid synthesis (hepatic 7α-hydroxylation activity) than 7α-hydroxy-4-cholesten-3-one alone under varying conditions in both rabbit models and in human beings. In analogy with 4β-hydroxycholesterol, 7α-hydroxy-4-cholesten-3-one is transported in lipoproteins, mainly in the LDL and HDL fractions [19]. Thus, the plasma lipoprotein status is important for the plasma concentration of 7α-hydroxy-4-cholesten-3-one.

As can be seen in fig. 1, the absolute concentrations of 4β-hydroxycholesterol after statin treatment were reduced. This is in accordance with a recently published study showing reduced 4β-hydroxycholesterol levels after statin treatment in 15 non-menopausal women with PCOS [20]. Weak cholesterol synthesis inhibition (fluvastatin 20 mg/day) resulted in a moderate reduction in plasma 4β-hydroxycholesterol, while treatment with a strong cholesterol synthesis inhibitor (atorvastatin 80 mg/day) resulted in a large reduction in plasma 4β-hydroxycholesterol. As shown in fig. 1, the 4β-hydroxycholesterol/cholesterol ratio was not changed after fluvastatin treatment and was slightly increased after strong inhibition of cholesterol synthesis by atorvastatin. Different studies have shown conflicting results regarding the effect of atorvastatin on CYP3A expression and activity [16,20–22]. However, since different doses and follow-up times as well as different types of patients have been used in these studies, it is difficult to draw any firm conclusion on this issue. In contrast, fluvastatin is not known to affect CYP3A activity and is mainly metabolized by CYP2C9 [23].

An in vitro experiment has shown that CYP3A4 is saturated with cholesterol already at a concentration of 100 μM [15]. Statin treatment had no effect on hepatic CYP3A4 mRNA levels in this cohort [16]. Together this indicates that during statin treatment, it is mainly the cholesterol-dependent lipoprotein transport capacity in the circulation that will determine the plasma 4β-hydroxycholesterol concentration rather than a direct effect on the hepatic CYP3A enzymes. Thus, our results suggest that the use of the 4β-hydroxycholesterol/cholesterol ratio rather than 4β-hydroxycholesterol alone is the preferred measure of CYP3A activity.

A limitation of this study is that we only have one marker of CYP3A and it would have been of interest to have another CYP3A activity marker to compare with. Unfortunately, no probe drugs such as midazolam or quinine had been given to the participants and no serum or urine was left from the study to analyse 6β-hydroxycortisol to cortisol ratio. Thus, additional studies with another CYP3A activity marker present are required to confirm the results from the present study. It would also have been of interest to genotype the individuals for CYP3A5. However, since 4β-hydroxycholesterol is formed from cholesterol by both CYP3A4 and CYP3A5 and thus reflects the sum of these activities, this would probably not affect the results.

In conclusion, the total plasma cholesterol level is an important determinant for the plasma 4β-hydroxycholesterol level.

Acknowledgements

Financial support was provided through the regional agreement on training and clinical research (ALF) between Karolinska Institutet and Stockholm County Council, unrestricted grants from Pfizer, Swedish Heart and Lung Foundation, Magnus Bergwalls Stiftelse and Karolinska Institutet.

References


© 2015 Nordic Association for the Publication of BCPT (former Nordic Pharmacological Society)


19 Gálman C, Angelin B, Rudling M. Bile acid synthesis in humans has a rapid diurnal variation that is asynchronous with cholesterol synthesis. Gastroenterology 2005;129:1445–53.


