

A comparison of 4 β -hydroxycholesterol : cholesterol and 6 β -hydroxycortisol : cortisol as markers of CYP3A4 induction

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WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- We have suggested that plasma 4 β -hydroxycholesterol may be used as an endogenous marker for cytochrome P450 3A4/5 (CYP3A)4/5 activity. We have shown that it can be used to monitor both induction and inhibition of CYP3A activity.

WHAT THIS STUDY ADDS

- Plasma 4 β -hydroxycholesterol : cholesterol was compared with another marker of CYP3A activity, 6 β -hydroxycortisol : cortisol in urine. Urine collections from volunteers treated with rifampicin for 2 weeks were analyzed for this ratio and compared to earlier determined plasma 4 β -hydroxycholesterol : cholesterol. The two markers gave similar induction ratios.

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AIM

To compare plasma 4 β -hydroxycholesterol : cholesterol with urinary 6 β -hydroxycortisol : cortisol as markers of cytochrome P4503A4 activity before and after treatment with rifampicin for 2 weeks.

METHOD

6 β -hydroxycortisol and cortisol were determined by liquid chromatography tandem mass spectrometry and 4 β -hydroxycholesterol was determined by gas chromatography–mass spectrometry in three groups of healthy volunteers.

RESULTS

Induction ratios for 6 β -hydroxycortisol : cortisol were 1.8, 3.9 and 4.5 for 20 mg day⁻¹, 100 mg day⁻¹ or 500 mg day⁻¹ of rifampicin, respectively. The corresponding ratios for 4 β -hydroxycholesterol : cholesterol were 1.5, 2.4 and 3.8.

CONCLUSIONS

Plasma 4 β -hydroxycholesterol : cholesterol gave similar induction ratios to urinary 6 β -hydroxycortisol : cortisol.

Introduction

Cytochrome P4503A (CYP3A) is an important enzyme family metabolizing both drugs and endogenous substances such as steroids. We have shown previously that

CYP3A4 converts cholesterol into 4 β -hydroxycholesterol [1] and suggested that plasma 4 β -hydroxycholesterol may be used as a phenotypic marker of CYP3A activity [2]. CYP3A4 also metabolizes cortisol to 6 β -hydroxycortisol, which has been used as an endogenous marker for CYP3A

activity. Since cortisol shows a pronounced diurnal variation, the ratio of 6 β -hydroxycortisol:cortisol in urine is used rather than 6 β -hydroxycortisol itself [3, 4]. We have previously studied 4 β -hydroxycholesterol as a marker of CYP3A-activity in healthy volunteers treated with rifampicin [5]. There is a weak but significant correlation between 4 β -hydroxycholesterol and cholesterol. Therefore, the 4 β -hydroxycholesterol to cholesterol ratio should be used rather than 4 β -hydroxycholesterol itself to correct for variations in cholesterol concentration during the study period [6]. In the present investigation we compared urinary 6 β -hydroxycortisol:cortisol to plasma 4 β -hydroxycholesterol:cholesterol determined previously [5].

Methods

Chemicals

6 β -hydroxycortisol, cortisol and cortisone were from Sigma-Aldrich Sweden AB, Stockholm, Sweden. $^2\text{H}_4$ -6 β -hydroxycortisol was from Toronto Research Chemicals Inc., North York, Canada and $^2\text{H}_4$ -cortisol was purchased from Larodan Fine Chemicals AB, Malmö, Sweden.

Calibration standards

Working solutions of calibrators were prepared from stock solutions (300 $\mu\text{mol l}^{-1}$ in methanol) by dilution with water to 700 nmol l^{-1} .

Quality control (QC) samples

Quality control samples were prepared from spiked human urine at two concentrations. The concentrations of 6 β -hydroxycortisol, cortisol and cortisone were 255, 89 and 127 nmol l^{-1} , respectively (concentration 1) and 933, 235 and 482 nmol l^{-1} (concentration 2).

Healthy volunteers

Twenty-four healthy Swedish volunteers were recruited to the study, 12 males and 12 females, as described previously [5]. All subjects possessed the CYP3A5*3/*3 genotype [5]. Urine was collected from 20.00 h to 08.00 h (12 h) before treatment and after 2 weeks of treatment with rifampicin. All volunteers gave their written informed consent to participate in the study. The study was approved by the Human Ethics Committee at Karolinska Institutet, Stockholm, Sweden.

Determination of 6 β -hydroxycortisol and cortisol in urine

6 β -hydroxycortisol, cortisol and cortisone in urine were determined by liquid chromatography tandem mass spectrometry (LC-MS/MS) using deuterium labelled 6 β -hydroxycortisol ($^2\text{H}_4$ -6 β -hydroxycortisol) and deuterium labelled cortisol ($^2\text{H}_4$ -cortisol) as internal standards.

Sample preparation

Urine (200 μl), QC samples or calibrators were diluted with an equal volume of water containing the internal

standards $^2\text{H}_4$ -6 β -hydroxycortisol (1000 nmol l^{-1}) and $^2\text{H}_4$ -cortisol (400 nmol l^{-1}). The diluted sample was applied to a 96-well solid phase extraction plate (Oasis HLB $\mu\text{Elution}$ Plate, Waters). After washing with 200 μl 5% methanol in water, analytes were eluted with 100 μl acetonitrile:isopropanol 2:3 (v/v) and diluted with 400 μl water. The samples were prepared on a Tecan Genesis RSP 150/TeVacs robot.

Analysis by LC-MS/MS

The samples (5 μl) were analyzed on a Waters Acquity-Quattro Premier LC-MS/MS system with a Waters UPLC BEH ShieldRP18 column (1.7 μm , 2.1 \times 150 mm) at 55°C. The mobile phase was a 7 min linear gradient of 5–50% acetonitrile in water with formic acid (0.1%), flow rate 0.400 ml min^{-1} and total run time 12 min. Retention times for 6 β -hydroxycortisol, cortisol and cortisone were 4.27, 6.27 and 6.30 min, respectively.

The mass spectrometer was operated in negative electrospray mode for 6 β -hydroxycortisol and positive mode for cortisol and cortisone. The transitions used were 347 \rightarrow 313 and 347 \rightarrow 125 (6 β -hydroxycortisol), 363 \rightarrow 121 and 363 \rightarrow 97 (cortisol) and 361 \rightarrow 163 and 361 \rightarrow 121 (cortisone). Transitions for the internal standards were 351 \rightarrow 128 ($^2\text{H}_4$ -6 β -hydroxycortisol) and 367 \rightarrow 121 ($^2\text{H}_4$ -cortisol).

Method validation

The linear range was 10–14 000 nmol l^{-1} for 6 β -hydroxycortisol and 5–14 000 nmol l^{-1} for cortisol and cortisone. Urine samples spiked with 0.8–7 times the original concentration (255, 89 and 127 nmol l^{-1} , respectively) gave recoveries of 90–97%. Urine samples spiked with 30–150 times the original concentration (933, 235 and 482 nmol l^{-1} , respectively) gave recoveries of 105–123%. The total relative standard deviations (CV) were between 5.0 and 9.2% ($n = 80, 117$ and 115 , respectively). The cortisol analysis is accredited according to SS-EN ISO 15189. Our laboratory participates in an external quality control programme for cortisol (UKNEQAS) on a regular basis.

Determination of 4 β -hydroxycholesterol in blood plasma

The results from the determination of 4 β -hydroxycholesterol in plasma were from a previous investigation [5]. 4 β -hydroxycholesterol was determined by combined gas chromatography-mass spectrometry as described in [1].

Determination of cholesterol in blood plasma

Cholesterol was determined by an enzymatic method, CHOD-PAPP, utilizing a Roche/Hitachi Modular instrument. The CV was 4% at 3 mmol l^{-1} .

Results

Determination of 6 β -hydroxycortisol : cortisol in urine from rifampicin treated volunteers

The urinary concentrations of 6 β -hydroxycortisol and cortisol were determined in three groups of volunteers (eight in each group) treated with rifampicin (20 mg day⁻¹, 100 mg day⁻¹ or 500 mg day⁻¹) for 14 days and the 6 β -hydroxycortisol : cortisol ratios were calculated. As shown in Figure 1, the ratios before treatment were similar, 6.3, 5.4 and 7.3 and increased after 14 days treatment to 10.5, 16.2 and 27.3, respectively. Three subjects, two receiving 20 mg day⁻¹ and one receiving 100 mg day⁻¹, showed a reduced 6 β -hydroxycortisol : cortisol ratio after 14 days of rifampicin treatment.

Plasma 4 β -hydroxycholesterol concentrations in rifampicin treated volunteers

The plasma 4 β -hydroxycholesterol concentrations in the volunteers were published previously [5]. In contrast to 6 β -hydroxycortisol : cortisol, the plasma concentration of 4 β -hydroxycholesterol increased in all volunteers after rifampicin treatment.

Calculation of induction ratios

The induction ratio was defined as 6 β -hydroxycortisol : cortisol after treatment divided by the 6 β -hydroxycortisol : cortisol before treatment. These induction ratios and the corresponding ratios for 4 β -hydroxycholesterol and quinine metabolic ratio published previously [5] are shown in Table 1. The ratio of 4 β -hydroxycholesterol : cholesterol should be used when the cholesterol concentration changes during a treatment period. There was a large interindividual variation in 6 β -hydroxycortisol : cortisol. The ratios determined before rifampicin treatment showed a 10-fold variation and the ratios after treatment showed a 15-fold variation. The corresponding variations for 4 β -hydroxycholesterol were three-fold and six-fold, respectively.

Discussion

The induction of CYP3A4 could be monitored by 6 β -hydroxycortisol : cortisol at all three doses of rifampicin.

Table 1

Induction ratios for 6 β -hydroxycortisol : cortisol, 4 β -hydroxycholesterol, 4 β -hydroxycholesterol : cholesterol and quinine metabolic ratio (MR) in volunteers treated for 2 weeks with 20, 100 or 500 mg rifampicin day⁻¹ (mean \pm SD, $n = 8$). Data on 4 β -hydroxycholesterol and quinine metabolic ratio (MR) are from a previous publication [5]

Rifampicin (mg day ⁻¹)	6 β -OH-cortisol : cortisol	4 β -OH-cholesterol	4 β -OH-cholesterol : cholesterol	Quinine MR
20	1.77 \pm 0.82	1.46 \pm 0.24	1.53 \pm 0.20	1.57 \pm 0.33
100	3.92 \pm 3.28	2.49 \pm 0.66	2.40 \pm 0.75	2.95 \pm 1.10
500	4.46 \pm 1.81	4.06 \pm 0.85	3.76 \pm 0.67	4.25 \pm 2.07

Compared with 4 β -hydroxycholesterol : cholesterol, the P values for the differences in the 6 β -hydroxycortisol : cortisol before and after treatment were higher [5] and in contrast to 4 β -hydroxycholesterol : cholesterol some individuals had lower ratios after treatment than before treatment (Figure 1). However, the induction ratios for 6 β -hydroxycortisol : cortisol and 4 β -hydroxycholesterol : cholesterol are similar and also similar to the metabolic ratio of the exogenous CYP3A marker quinine (Table 1, data on 4 β -hydroxycholesterol and quinine are from [5]). An advantage with 4 β -hydroxycholesterol is that only a single blood sample is required for the determination while a urine collection over at least 12 h is necessary when using 6 β -hydroxycortisol : cortisol. The long half-life of 4 β -hydroxycholesterol [7] results in small variations in concentration, but excludes this marker in short term studies.

In conclusion, similar estimates for the induction of CYP3A4 following rifampicin treatment were obtained using either urinary 6 β -hydroxycortisol : cortisol or plasma 4 β -hydroxycholesterol : cholesterol.

Competing Interests

K.P. Kanebratt is an employee of AstraZeneca. Unrestricted financial support was provided by AstraZeneca. This has not influenced the design or the interpretation of results of this study. All authors have completed the Unified Competing Interest form at http://www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare no support from any organization for the submitted work (YMA, HN, AL-S, KPK, KW, UD had support (grant) from AstraZeneca for the submitted work and no financial relationships with any organizations that might have an interest in the submitted work in the previous 3 years (YMA, HN, AL-S, KPK, KW). UD received support (grant, consultancy) from AstraZeneca in the previous 3 years and no other relationships or activities that could appear to have influenced the submitted work (YMA, HN, AL-S, KPK, KW, UD).

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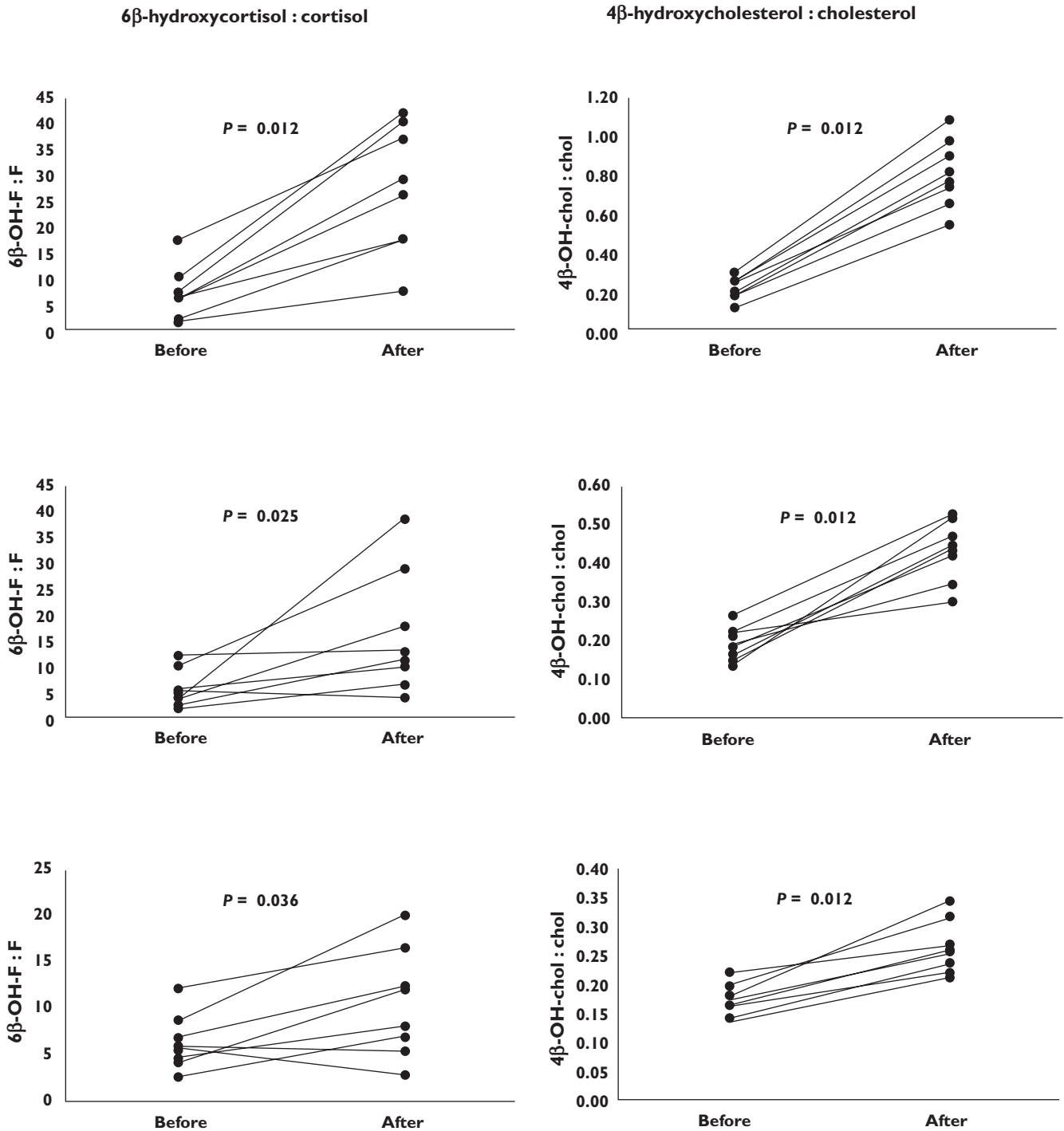


Figure 1

Urinary 6β-hydroxycortisol :cortisol and plasma 4β-hydroxycholesterol :cholesterol ($\times 10^4$) in adult healthy volunteers before and after 2 weeks of rifampicin treatment (500 mg day⁻¹, upper panels; 100 mg day⁻¹, middle panels; 20 mg day⁻¹, lower panels). The 6β-hydroxycortisol :cortisol ratios were determined in 12 h urine collections. *P* values from analysis using Wilcoxon matched pairs test. 6β-OH-F: 6β-hydroxycortisol; F: cortisol; 4β-OH-chol: 4β-hydroxycholesterol; chol: cholesterol

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