URINARY 6β-HYDROXYCORTISOL EXCRETION IN RHEUMATOID ARTHRITIS

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SUMMARY
The objective was to analyse whether the activity of the cytochrome P450 isoenzyme CYP3A4 is altered by disease activity of rheumatoid arthritis (RA). Urinary 6β-hydroxy cortisol excretion, expressed as a fraction of the urinary creatinine output, was measured in 21 patients with RA treated with three different disease-modifying anti-rheumatic drugs (DMARDs) over 24 weeks. There were no correlations between urinary 6β-hydroxy cortisol/creatinine (6β-OHC/Creat) ratio and measurements of disease activity such as plasma viscosity, Ritchie articular index and early morning stiffness. In addition, the three DMARDs sulphasalazine, sodium aurothiomalate and penicillamine, smoking and the intake of various CYP3A4 substrates had no consistent detectable effect on the 6β-OHC/Creat ratio. There is no evidence that the dosage of drugs metabolized by the CYP3A4 isoenzyme needs to be adjusted for disease activity in RA.

KEY WORDS: 6β-Hydroxycortisol, CYP3A4, Rheumatoid arthritis, Disease activity, DMARD.

Drugs used to treat rheumatoid arthritis (RA) come from different chemical backgrounds and are metabolized in different ways. Thus, metabolism, involving different enzyme systems of biotransformation, is affected by many variables such as age, gender, diet, smoking habits, environmental factors and genetic constitution [1-3]. Some of the enzyme systems involved, such as the cytochrome P450, can be induced and inhibited by a wide range of chemically unrelated substances, including drugs [2]. This may be followed by change in the ratio of drug efficacy to toxicity, especially for drugs with a small therapeutic ratio and with long-term effect, making an alteration in dosage schedule necessary. Drug metabolism is also affected by hepatic [4] and extrahepatic [5] diseases such as diabetes mellitus, thyroid disease, cardiopulmonary dysfunction and malignancy. Inflammation itself may also affect the rate of drug metabolism [5]. Acute viral infections and influenza vaccination can impair cytochrome P450-dependent oxidative drug metabolism, leading to toxicity from theophylline, phenytoin or warfarin therapy [6-9]. Interferon and interferon inducers exert an indirect, and interleukin-1 and tumour necrosis factor, a direct inhibitory effect on oxidative drug-metabolizing enzymes [10-12].

Effective clinical probes are necessary to unravel these many confounding factors before advice for an optimum dosage can be given, both in healthy subjects and especially in subjects suffering from systemic inflammatory diseases such as RA, where disease activity may in itself alter drug metabolism. The use of probe drugs offers one option, but an alternative is to measure changes in the disposition of an endogenous substance which is metabolized by the hepatic mixed function oxidase system. Urinary 6β-hydroxycortisol (6β-OHC), normally a minor metabolite of cortisol, has been successfully employed in this way for the assessment of enzyme induction by drugs such as phenobarbitone, rifampicin, antipyrine, phenytoin and carbamazepine [13-16]. Its urinary output is also increased by occupational exposure to the organochlorine insecticide DDT [17], but is independent of cigarette smoking [18]. 6β-OHC urinary excretion is reduced with the use of cimetidine [15] and of the anti-fungal agent ketoconazole [19] and in hyperthyroidism [20]. Twenty-four hour excretion is highly correlated with the expression of the human hepatic cytochrome P450 3A4 [21]. There is evidence that the glucocorticoid-inducible CYP3A subfamily is responsible for the metabolism of a number of clinically important drugs, including nifedipine, the benzodiazepines midazolam and triazolam, erythromycin and, of particular relevance in RA, codeine and cyclosporin [22, 23]. 6β Hydroxylation is the major route of excretion for testosterone, androsterone and progesterone [24]. Excretion thus varies between females and males [25]. Apart from this sexual dimorphism, there is an apparent unimodal distribution of urinary 6β-OHC excretion [26]. Change due to circadian variation can be corrected either by using 24 h urine collections or by expressing results as a 6β-OHC/free cortisol ratio [16, 27]. Variation of 6β-OHC excretion due to incomplete urine collection is corrected by expressing results as a 6β-OHC/urinary creatinine ratio (6β-OHC/Creat).

We have investigated the relevance of 6β-OHC excretion in RA as an index of metabolism when the disease is treated with different disease-modifying anti-rheumatic drugs (DMARDs).

PATIENTS AND METHODS
Patients with RA (ARA criteria) [28] and disease activity sufficient to justify the use of DMARDs were recruited [plasma viscosity (PV) > 1.72 mPa or Ritchie...
articular index (AI) [29] > 16 or early morning stiffness (EMS) > 1 h]. Patients with a history of or with laboratory evidence of hepatitis due to excessive alcohol intake, infectious, autoimmune or storage diseases were excluded.

Patients were allocated either to sulphasalazine (SASP), sodium aurothiomalate (Gold) or d-penicillamine (DPA) according to clinical criteria by the investigating physician blinded to the results of 6β-OHC excretion. Standard dosage regimens were used [30]. All subsequent dosage adjustments, mandatory due to toxicity, and all concomitant drug therapy, particularly drugs that might interfere with cytochrome P450 metabolism, were meticulously recorded. Moreover, all concomitant drug therapy remained in constant dosage throughout the study, unless a change was necessary because of toxicity or on ethical grounds. No patient received systemic steroid therapy or intra-articular steroid injections during the 3 months before the investigation.

At 0, 12 and 24 weeks, disease activity of RA and safety of the DMARD treatments were assessed including the following parameters: AI [29], EMS (minutes), PV [31], full blood count, liver function tests (serum aspartate aminotransferase, total alkaline phosphatase, gamma-glutamyl transpeptidase (GGT) [32]) and renal function tests (serum creatinine, urea and urine analysis for protein, white and red blood cells). In conjunction with each clinic visit, a 24 h urine collection was obtained. After measurement of total volume and careful mixing, aliquots were taken and kept at −20°C until analysed. Urinary 6β-OHC was measured by radioimmunoassay according to Park [25, 33] and urinary creatinine by a standard spectrophoto-
metric assay [34]. Analytical variability of urinary 6β-OHC (coefficient of variation) was 5.5 and 10.9% for samples with concentrations of 400 and 50 pg/ml, respectively. Physiological variability in healthy volunteers on two successive days was calculated to be 2.3% [14, 35].

Statistical analyses included median, interquartile range (IQR), Spearman rank correlation (r), Wilcoxon signed rank test for differences between paired observations and Wilcoxon rank sum test for comparison of two groups [36]. The significance level was set at \( P = 0.05 \) with two-sided testing. In the case of multiple statistical comparisons, Bonferroni adjustments were used per parameter. PV was considered as the primary reference parameter of disease activity. The power to detect a 25% difference between week 0 and week 24 was calculated to be 0.96 (6βOH/Creat), 0.64 (GGT), 1.00 (PV), 0.70 (AI) and 0.12 (EMS), respectively.

Ethical committee approval was obtained from the Ethics Committee of the Leeds Western Health Authority and all patients gave informed consent.

**RESULTS**

Twenty-one patients, three males and 18 females, suffering from RA of at least 6 months duration (median 10 yr, IQR 4–19) were studied. The median age was 56 yr (IQR 51–67) and median weight was 60 kg (IQR 56–79). Five patients were smoking 5–30 cigarettes/day (median 20 cigarettes), while 16 patients were non-smokers. Twelve out of the 21 patients (57%) had been treated with a conventional DMARD in the past [SASP (n = 3), DPA (n = 3), Gold (n = 2), hydroxychloroquine (n = 2), auranofin (n = 1) and methotrexate (MTX) (n = 1)]. All 12 patients started a different DMARD, allowing a treatment interval of at least 3 months for Gold, 1 month for MTX and 1 week for the remainder.

At the beginning of the study, all 21 patients were allocated to a new DMARD treatment. Seven patients were started on SASP, nine on Gold and five on DPA. Eight patients were given substrates of the CYP3A4 isozyme that might influence the 6β-OHC output, such as nifedipine (one patient taking 80 mg/day from week 0 to 24), codeine (one patient taking 24 mg/day at week 12 and one 48 mg/day and 32 mg/day at week 12 and 24, respectively) and dihydrocodeine (two patients taking between 20 and 40 mg/day from week 0 to 24, one patient with 30 mg/day at week 12, one patient with 120 mg/day at week 0 and finally one with 120 mg/day and 60 mg/day at week 12 and 24, respectively). One patient was taking quinalbarbitone 100 mg/day from week 0 to 24, a well-established inducer of cytochrome P450. All other drugs used were felt to be unlikely to influence the CYP3A4 isozyme.

At the beginning of the study, median 6β-OHC excretion was 274 μg/24 h (IQR 206–450) and median baseline creatinine clearance was 100 ml/min (IQR 66–134). During the 24 weeks investigated, there was

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**TABLE 1**

Ratio of urinary 6β-hydroxycortisol/creatinine in 24 h urine collections, gamma-glutamyl transpeptidase (GGT), plasma viscosity (PV), Ritchie articular index (AI) and early morning stiffness (EMS) at weeks 0, 12 and 24 in 21 patients with rheumatoid arthritis after the introduction of a disease-modifying anti-rheumatic drug. Medians, interquartile ranges (IQR) and statistical differences compared to baseline values are given.

<table>
<thead>
<tr>
<th>Week 0</th>
<th>Week 12</th>
<th>Week 24</th>
</tr>
</thead>
<tbody>
<tr>
<td>43 [30–58]</td>
<td>35 n.s. [21–57]</td>
<td>42 n.s. [33–54]</td>
</tr>
<tr>
<td>1.85 [1.74–1.94]</td>
<td>1.75** [1.71–1.88]</td>
<td>1.73*** [1.65–1.83]</td>
</tr>
<tr>
<td>120 [40–240]</td>
<td>60 n.s. [35–180]</td>
<td>30 n.s. [10–120]</td>
</tr>
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</table>

* \( P < 0.05 \); ** \( P < 0.02 \); *** \( P < 0.002 \), n.s. = non-significant with Bonferroni’s adjustment per parameter (Wilcoxon signed rank test).
no significant change either in 6β-OHC excretion or in creatinine clearance.

Variation due to incomplete urine collection was corrected by expressing results as a ratio of urinary 6β-OHC to urinary creatinine (6β-OHC/Creat). Table I summarizes 6β-OHC/Creat ratio, GGT, PV, AI and EMS. After 12 and 24 weeks of DMARD treatment, significant decreases of PV and AI were observed, whether correcting for multiple comparisons per parameter or not. In contrast, 6β-OHC/Creat ratio, GGT and EMS remained unchanged during the 24 weeks follow-up. No correlations were found between 6β-OHC/Creat ratio at baseline and GGT [rs = −0.07, non-significant (n.s.)], PV (rs = −0.02, n.s.), AI (rs = 0.17, n.s.) and EMS (rs = 0.30, n.s.).

Similarly, no correlations were revealed between intraindividual change in the 6β-OHC/Creat ratio from week 0 to 24 and GGT (rs = −0.13, n.s.), PV (rs = 0.05, n.s.), AI (rs = −0.33, n.s.) and EMS (rs = 0.08, n.s.).

There were no correlations between 6β-OHC/Creat ratio and age or weight (rs = 0.18, n.s. and rs = 0.14, n.s., respectively). Slightly higher values of 6β-OHC/Creat ratios were found in females compared to males [week 0: median 35 (IQR 22–52) μg/mmol in four patients on substrates compared to 40 (30–62) in 16 patients without substrates, n.s.; week 12: 36 (25–58) in seven patients on substrates compared to 35 (18–60) in 13 patients without substrates; week 24: 30 (15–35) in five patients on substrates compared to 44 (36–55) in 15 patients without substrates, P < 0.05]. One patient on quinalbarbitone had 6β-OHC/Creat ratios in the upper part of the IQR of the 21 patients studied (week 0: 51 μg/mmol, week 12: 46 μg/mmol, week 24: 57 μg/mmol).

Figure 1 illustrates the time course of the 6β-OHC/Creat ratios in the three DMARD treatment groups studied. At baseline, no significant differences were found between patients treated with SASP, Gold or DPA. In addition, no significant modulating effect of the three DMARDs on the 6β-OHC/Creat ratios over 24 weeks was apparent.

**DISCUSSION**

In the present investigation, the activity of the cytochrome P450 isoenzyme CYP3A4 was assessed by determining the urinary 6β-OHC/Creat ratio. Several assumptions have to be considered when this ratio is used as a quantitative measure for the activity of CYP3A4. First, the muscle mass has to be constant within each patient during the study period in order to exclude an unwarranted influence of urinary creatinine excretion on that ratio. We can exclude a changing muscle mass during the study period for the patients analysed, since the 24 h urinary creatinine excretion did not change as a function of time. Second, other metabolic routes or the non-renal elimination of 6β-OHC have to remain constant during the entire study and, third, cortisol production should not change as a function of time. We have no evidence that the second and third assumptions are valid. However, the experience with model compounds such as barbiturates, rifampicin, antipyrine, phenytoin and...
carbamazepine [13–16] clearly revealed that despite the absence of validation of these assumptions, the urinary ratio of 6β-OHC/Creat is a useful parameter for measurement of the activity of the CYP3A4 isoenzyme.

Subjects with an increased 6β-OHC/Creat ratio metabolize xenobiotics cleared by this enzymatic pathway faster than subjects with lower excretion of this cortisol metabolite. This has been clearly demonstrated for the exogenous glucocorticoid prednisolone [25]. Since the fractional clearance attributable to the isoenzyme CYP3A4 varies from compound to compound and is unknown in humans, it is hard to predict on the basis of the indirect activity of CYP3A4 how it affects the exact therapeutic dose finding of the xenobiotic. However, one might predict that subjects with a CYP3A4 isoenzyme activity enhanced by > 25% have a clinically significantly enhanced clearance of xenobiotics exhibiting a fractional metabolic clearance attributable to that isoenzyme.

There was a failure of the 6β-OHC/Creat ratio to correlate with disease activity as assessed by PV, AI and EMS. In addition, there was a lack of correlation with GGT representing a membrane-bound liver enzyme related to a variety of laboratory and clinical markers of disease activity, including PV, C-reactive protein, platelets, AI and EMS [37]. We are well aware that the number of patients studied is small in view of the many factors which can confound the results, such as age, gender, environmental agents and drugs. The lack of an obvious influence of disease activity on the 6β-OHC/Creat ratio as a measure of the CYP3A4 enzyme activity has to be interpreted with caution. It is certainly possible that a minor effect was missed. However, when analysing intraindividual changes over time, most factors remain constant. The failure of intraindividual change of disease activity to correlate with intraindividual change of 6β-OHC/Creat casts doubt on the influence to an extent that is clinically relevant. From these results, we find no evidence to suggest that the dosage of drugs metabolized by the CYP3A4 isoenzyme needs to be adjusted to disease activity.

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