Inhibition of caffeine metabolism by 5-methoxypsoralen in patients with psoriasis

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Eight patients with psoriasis were given 200 mg caffeine orally with or without 1.2 mg kg⁻¹ of 5-methoxypsoralen. Blood and urine samples were collected over a 2-day period. During 5-methoxypsoralen coadministration, the apparent volume of distribution of caffeine remained unchanged, but oral clearance (CL_{p.o.}) decreased from 9.5 ± 3.8 (mean ± s.d.) to 3.2 ± 0.5 l h⁻¹ ($P < 0.01$). The area under the plasma concentration-time curve (AUC) increased from 24 ± 9 to 73 ± 29 mg l⁻¹ h ($P < 0.001$). This decrease in CL_{p.o.} with increased AUC was consistent with a CYP1A2-dependent inhibition of caffeine *N*-demethylation which was further supported by significant decreases in the (AFMU + 1U + 1X)/17U and (AFMU + 1U + 1X)/17X urinary metabolic ratios.

Keywords 5-methoxypsoralen caffeine metabolism inhibition CYP1A2

Introduction

The metabolism of caffeine [1] and theophylline [2] is inhibited by 8-methoxypsoralen (8-MOP). Another psoralen, 5-methoxypsoralen (5-MOP), has been proposed for treatment of psoriasis as an alternative to 8-MOP since it has the same efficacy, but shows better tolerance [3–5]. This study was carried out to analyse the effect of 5-MOP on the metabolism of caffeine in patients with psoriasis. Caffeine (1,3,7-trimethylxanthine or 137X) can be used to assess microsomal CYP1A2, cytosolic xanthine oxidase (XO) and polymorphic *N*-acetyltransferase (NAT2) activities [6–10].

Methods

Subjects

The study was conducted on six men and two women with psoriasis. Three of them were smokers and none of the women was taking oral contraceptives. Mean (range) age was 37 (20–76) years and body weight was 67 (54–95) kg. The study was approved by the Ethics Committee of Besançon University Hospital and informed consent was obtained from all the subjects.

Study design

The subjects, who were not taking any medication at the time of the study, were requested to abstain from food and beverages containing psoralens (e.g. figs, celery), methylxanthines (coffee, tea, cola, chocolate) or any soft drinks 48 h before and during the study.

On two occasions separated by a 1-week interval, the subjects were given 200 mg of caffeine *per os*, either alone or in combination with 1.2 mg kg⁻¹ of 5-MOP. The order of drug administration was randomized. Blood samples were collected in 5 ml heparinized tubes (Beckton Dickinson, Meylan, France) through an indwelling venous catheter, before caffeine intake and 10, 20, 30, 40, 50 min, 1, 2, 3, 4, 6, 8, 12, and 24 h after dosing. When 5-MOP was given with caffeine, three extra samples were collected 30, 36 and 48 h after dosing. Samples were immediately centrifuged (3000 r.p.m. 5 min) and plasma was stored in the dark at –20° C until assay.

Abstention from methylxanthine intake was verified by analysing urine just before drug intake. Urine was then collected in two batches, 0–24 h and 24–48 h after dosing, and into bottles containing 1 ml of 6 N HCl. The volume of urine was measured and two aliquots of each sample were transferred into glass tubes containing 20 mg ascorbic acid ml⁻¹ of urine. The tubes were frozen and kept at –20° C until analysis, which was

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performed less than 2 days after the end of each experiment.

Assays

Plasma caffeine concentrations were measured by the h.p.l.c. method of Campbell *et al.* [7] and those of 5-MOP according to Herfst *et al.* [11], using 8-MOP as the internal standard. Urinary concentrations of caffeine and its metabolites [5-acetylamino-6-formylamino-3-methyluracil (AFMU), 1-methylurate (1U), 1,3-dimethylurate (13U), 1,7-dimethylurate (17U), 1-methylxanthine (1X), 3-methylxanthine (3X), 7-methylxanthine (7X), 1,3-dimethylxanthine (theophylline or 13X), 1,7-dimethylxanthine (paraxanthine or 17X), 3,7-dimethylxanthine (theobromine or 37X)] were measured by h.p.l.c. as described by Grant *et al.* [6]. Coefficients of variation were 3.7% and 4.0% for caffeine and 5-MOP measured in plasma and 2.3–6.2% for urinary caffeine metabolites, respectively.

Pharmacokinetic analysis and metabolic ratios

Maximum concentration ($C_{\max}$), time to reach $C_{\max}$ ($t_{\max}$), area under the plasma concentration-time curve (AUC), oral clearance ($CL_{p.o.}$) and volume of distribution (V_d), were calculated using a SIPHAR pharmacokinetic package (SIMED, Créteil, France) for both caffeine and 5-MOP. The following urine ratios were calculated and used as indices of specific enzyme activities [10]: (AFMU+1U+1X)/17U (CYP1A2 activity), (AFMU+1U+1X)/17X (17X 7-*N*-demethylase (7NDM) activity), 17U/17X (17X C8-hydroxylase (8OH) activity), AFMU/1X (*N*-acetyltransferase (NAT2) activity) and 1U/1X (xanthine oxidase (XO) activity).

Statistical analysis

Pharmacokinetic parameters of caffeine, molar amounts of urinary caffeine metabolites, and urinary metabolic ratios, with or without 5-MOP coadministration were compared using Statistical Package for the Social Sciences (SPSS, Chicago, IL, USA). The Student's *t*-test for paired data after log transformation was used, except for $t_{\max}$ and urinary metabolic ratios which were analysed by the Wilcoxon signed rank test for paired data. Results are expressed as mean ($\pm$ s.d.) except for $t_{\max}$ which is expressed as median (range). Differences were regarded as significant when $P < 0.05$.

Results

Following 5-MOP intake, the AUC of caffeine was increased by threefold, from 24 ± 9 to 73 ± 29 mg l⁻¹ h, $P < 0.001$. Caffeine plasma concentrations decreased with time as a convex curve, before reaching a log linear slope (Figure 1). As a consequence, $CL_{p.o.}$ decreased

Figure 1 Mean plasma concentrations of 5-MOP (Δ) and caffeine without ($\square$) and with ($\blacksquare$) 5-MOP coadministration in psoriatic patients. $n=7$ for 5-MOP since concentrations were below the limit of detection in one subject; $n=8$ for caffeine.

from 9.5 ± 3.8 to 3.2 ± 0.5 l h⁻¹, $P < 0.01$. Values of $t_{\max}$ and V_d of caffeine were not affected by 5-MOP coadministration.

After 5-MOP intake, caffeine urinary elimination during the first 24 h was increased from 0.001 ± 0.002 to 0.016 ± 0.013 mmol 24 h⁻¹, $P < 0.05$. Elimination of 1U, 1X, AFMU, 7X, 17X, 3X and 13U was significantly decreased (data not shown). 7NDM and CYP1A2 indices were significantly decreased during the first 24 h of the test (Figure 2).

Discussion

Caffeine pharmacokinetic parameters are in agreement with those obtained by Brazier *et al.* [12] and Mays *et al.* [1], but those of 5-MOP are different from the results published by Stolk *et al.* [13]. In the present study the values of $t_{\max}$, $C_{\max}$ and AUC for 5-MOP are 6.0 (range 3.0–8.0) h, 0.03 ± 0.01 mg l⁻¹ and 0.38 ± 0.15 mg h l⁻¹ respectively, whereas the values reported by Stolk *et al.* [13] were 3.2 ± 0.8 h, 0.41 ± 0.10 mg l⁻¹ and 0.92 ± 0.46 mg h l⁻¹, respectively. Differences might be due to a reduced sampling period which was 5 h in the quoted report, compared with 48 h in our study, and to substantial interindividual variability observed in both studies.

Gastrointestinal absorption of caffeine is rapid and complete [14]. Therefore, it is unlikely that the observed increase in caffeine AUC was due to an increase in the absorbed fraction when 5-MOP was coadministered. Further evidence for the lack of any effect on absorption is the similarity in $t_{\max}$ observed with and without 5-MOP (Figure 1). Caffeine clearance is mainly metabolic, since less than 2% of a 200 mg oral dose is excreted unchanged in urine in 24 h [7]. Therefore, the increase in AUC observed after 5-MOP was the consequence of a decrease in clearance.

A semilogarithmic plot of caffeine concentrations after

Figure 2 Ratios of the molar concentrations of urinary caffeine metabolites as indices of polymorphic *N*-acetyltransferase (NAT2), 7-*N*-demethylase (7NDM), CYP1A2 (1A2), C8-hydroxylase (8OH) and xanthine oxidase (XO) activities. Results without (▨) and with (▧) 5-MOP coadministration, over 0–24 h (A) and 24–48 h (b) in patients with psoriasis ($n=8$). *: $P < 0.05$ when compared with caffeine alone.

5-MOP (Figure 1) shows a fractional rate of elimination slower at high than at low concentrations (upward convex curve). In light of previous suggestions that the 8-isomer of methoxypsoralen is a suicide inhibitor [15, 16], this type of kinetic profile is consistent with suicide inhibition involving the inactivation of a proportion of CYP1A2 by 5-MOP reactive metabolite(s). Thus, caffeine metabolism would then become rapidly saturated. Twenty-four hours later, newly synthesized CYP1A2 would lead to a return of caffeine clearance back to initial values.

Dose dependent kinetics of caffeine have been reported in humans by Cheng *et al.* [17] and Denaro *et al.* [18], and are probably the consequence of saturation of the formation of paraxanthine and possibly others metabolic pathways. Thus, the fractional rate of caffeine elimination following 5-MOP coadministration is equally consistent with a capacity-limited metabolism as a consequence of the competitive inhibition of caffeine metabolism which would cause saturation at a lower plasma concentration. However, in view of previous studies on the inhibitory effect of psoralens on drug metabolism [1, 15, 16, 19], competitive inhibition is unlikely.

5-MOP selectively decreased the (AFMU + 1U + 1X)/17U and (AFMU + 1U + 1X)/17X ratios, which are both dependent of CYP1A2 activity [9, 10]. The inhibition of CYP1A2 activity by 5-MOP has been observed by Tassaneeyakul *et al.* [19] using cDNA-expressed enzymes and human liver microsomes. The other indexes of enzyme activity were not influenced by 5-MOP. This shows that, at therapeutic doses of 5-MOP used during PUVA therapy of psoriasis (1.2 mg kg^{-1}), the CYP1A2-dependent metabolism of caffeine is selectively inhibited.

Some of the adverse effects observed in psoriatic patients during methoxypsoralen treatment such as nervousness, insomnia or excitation [20, 21] might be explained by unusually high caffeine plasma concentrations, despite normal dietary consumption. This has been observed with other drugs such as idroclamide [12] and phenylpropanolamine [22]. Advice on the need to decrease coffee consumption during PUVA

therapy might be given to avoid the potential risk of side effects. Furthermore, the inhibition of CYP1A2 activity by 5-MOP should also be considered when other potent inhibitors of this enzyme, such as furafylline [23, 24], fluvoxamine [25] and oral contraceptives [26] are prescribed at the same time.

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