Variability of cytochrome P450 1A2 activity over time in young and elderly healthy volunteers

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Aims To assess the age-associated changes over time of plasma paraxanthine/caffeine (PAX/CAF) ratios used as a probe for CYP1A2 activity.

Methods Intraindividual and interindividual variabilities in PAX/CAF ratio were compared by phenotyping with caffeine, 16 young and 16 elderly healthy subjects on five occasions.

Results PAX/CAF ratio variability was comparable regardless of age (intraindividual CV: 17.6 ± 6% and 16.2 ± 5.9%, interindividual CV: 48.1 ± 2.9% and 42.7 ± 3.6% in young and elderly, respectively). The PAX/CAF ratio was lower in elderly than in young subjects (95% CI for the difference: 0.004, 0.32) but the difference was not significant in nonsmokers compared separately.

Conclusions The variability over time of the PAX/CAF ratio is not influenced by age.

Keywords: caffeine, cytochrome P450 1A2, human

Introduction

CYP1A2 is responsible for the metabolism of several drugs [1]. Measurement of caffeine urinary metabolic ratios [2], the [^{13}C]-caffeine breath test [3] and the paraxanthine/caffeine (PAX/CAF) ratio in plasma and saliva have been proposed for CYP1A2 phenotyping [4, 18]. Individual variability in CYP1A2 activity over time has been observed when using caffeine urinary metabolic ratios [2, 5], which is partly due to environmental factors [5] but also to variation in the urinary flow rate [6].

It has been shown that the PAX/CAF ratio determined in plasma is a more reliable index for assessment of CYP1A2 activity [4, 18]. However, intraindividual variability in the ratio determined in plasma has not been reported. Such variability is important in the interpretation of drug interaction studies. Moreover, studies of CYP1A2 activity have generally been performed in young volunteers whereas therapeutic drug use of drugs is more common among older age groups, in whom variability in CYP1A2 activity has not been established.

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The aim of the study was to compare the intraindividual and interindividual variability in plasma PAX/CAF ratio among young and elderly healthy volunteers over a 12 week period.

Methods

The study was approved by the Pitié Salpétrière ethics Committee. All subjects gave their written informed consent before inclusion. Sixteen subjects per group were needed to detect a 30% difference in plasma PAX/CAF ratios between young and elderly subjects [8] ($\alpha = 0.05$, $\beta = 0.20$). Sixteen healthy young (10 men and 6 women, aged 25 ± 0.3 years) and elderly subjects (6 men and 10 women, aged 70 ± 1.7 years) were studied. All subjects had a physical examination and underwent routine laboratory screening tests before entry. Any subject who had a body mass index ≥ 28 kg m^{-2}, regularly consumed alcohol (> 20 g day^{-1}), or had any treatment known to influence CYP1A2 activity within 1 month before or during the study, (including oral contraceptives in women) was excluded. The subjects were asked to maintain a stable diet throughout the study.

Phenotyping procedure

Subjects were requested to avoid methylxanthine-containing foods and beverages, 24 h before and during

each test, which consisted of the oral administration of 140 mg caffeine prepared from instant coffee (Nescafe, Nestlé) together with a venous blood sample taken 5 h later [8]. Plasma was stored at -20° C until analysis. The caffeine test was performed on five occasions, 3 weeks apart over a period of 12 weeks.

CAF and PAX in plasma were analysed by h.p.l.c. [19]. The detection and quantification limits for both compounds were 15 and 50 ng ml^{-1}, respectively. Standard curves were linear (correlation coefficients >0.999) over the range 50–5000 ng ml^{-1}. Coefficients of variation (CV) for within-day and between-day analyses were 7.4%, 7.4% and 7.5%, 12.7% for CAF and PAX, respectively.

Statistical analysis

The intraindividual and interindividual variability of metabolic ratios were compared by Friedman and Wilcoxon tests, respectively.

Results

All subjects attended the five phenotyping visits.

Intraindividual and interindividual variability in the PAX/CAF ratio

Table 1 shows the mean metabolic ratios from the five phenotyping tests. PAX/CAF ratio did not significantly

Table 1 Intraindividual variability in the PAX/CAF ratio in young and elderly healthy subjects.

	PAX/CAF ratio (MR)	*CV (%)*	*Lowest MR*	*Highest MR*	*Highest/lowest MR*
Young subjects					
1	0.36 ± 0.09	25.7	0.25	0.49	1.96
2§	0.84 ± 0.16	18.9	0.62	1.03	1.66
3	0.42 ± 0.05	11.0	0.37	0.49	1.32
4§	0.98 ± 0.26	26.5	0.63	1.35	2.14
5§	0.72 ± 0.17	23.7	0.42	0.83	1.98
6	0.41 ± 0.09	21.7	0.31	0.54	1.74
7	0.20 ± 0.04	21.0	0.16	0.25	1.56
8§	0.49 ± 0.09	18.2	0.41	0.61	1.49
9	0.23 ± 0.05	21.4	0.16	0.29	1.81
10	0.4 ± 0.06	15.3	0.33	0.49	1.48
11§	0.87 ± 0.16	18.8	0.64	1.03	1.61
12	0.43 ± 0.09	19.9	0.31	0.52	1.68
13	0.58 ± 0.05	8.0	0.52	0.64	1.23
14	0.31 ± 0.02	6.9	0.29	0.34	1.17
15	0.45 ± 0.06	12.7	0.36	0.5	1.39
16	0.69 ± 0.08	12.3	0.58	0.77	1.33
All subjects	0.52 ± 0.25	17.6 ± 6.0*			1.60 ± 0.28
Elderly subjects					
1§	0.39 ± 0.04	10.4	0.35	0.44	1.26
2	0.58 ± 0.09	15.2	0.47	0.7	1.49
3	0.23 ± 0.03	15.1	0.20	0.29	1.45
4	0.32 ± 0.03	8.6	0.29	0.36	1.24
5	0.15 ± 0.03	20.5	0.11	0.19	1.73
6	0.37 ± 0.12	32.9	0.29	0.58	2.00
7	0.27 ± 0.03	10.2	0.25	0.32	1.28
8	0.34 ± 0.06	16.1	0.25	0.39	1.56
9	0.24 ± 0.05	21.0	0.20	0.33	1.65
10§	0.53 ± 0.09	16.9	0.44	0.62	1.41
11§	0.42 ± 0.08	18.5	0.33	0.52	1.58
12	0.50 ± 0.10	20.3	0.35	0.62	1.77
13	0.10 ± 0.01	10.7	0.09	0.12	1.33
14	0.46 ± 0.06	14.2	0.38	0.53	1.39
15	0.23 ± 0.03	13.4	0.20	0.28	1.4
16	0.32 ± 0.05	15.4	0.26	0.39	1.5
All subjects	0.34 ± 0.14	16.2 ± 5.9*			1.50 ± 0.21

Data are expressed as mean value ± s.d. MR is the mean value of five consecutive measures over a 12 week period. *Intraindividual CV was similar among young and elderly subjects. §smokers.

Table 2 Interindividual variability in CYP1A2 activity in young and elderly healthy subjects.

	PAX/CAF ratio (MR)	*Coefficient of variation (%)*	*Lowest MR*	*Highest MR*	*Highest/lowest MR*
Young subjects					
Test 1	0.56 ± 0.29	52.3	0.16	1.35	8.44
Test 2	0.51 ± 0.25	48.0	0.21	1.05	5.00
Test 3	0.49 ± 0.23	46.3	0.16	1.03	6.44
Test 4	0.51 ± 0.26	50.2	0.25	1.03	4.12
Test 5	0.55 ± 0.24	43.9	0.24	0.99	4.13
Mean ± s.d.		48.1 ± 2.9*			5.62 ± 1.64
Elderly subjects					
Test 1	0.33 ± 0.12	37.9	0.12	0.52	4.33
Test 2	0.32 ± 0.13	40.4	0.10	0.55	5.50
Test 3	0.36 ± 0.16	44.0	0.10	0.62	6.20
Test 4	0.35 ± 0.17	48.6	0.10	0.70	7.00
Test 5	0.35 ± 0.15	42.6	0.09	0.62	6.89
Mean ± s.d.		42.7 ± 3.6*			5.98 ± 0.99

Data are expressed as mean value ± s.d. The metabolic ratio is the mean value of five consecutive measures over a 12 week period. The lowest and the highest measure at each test are presented with the ratio. *Interindividual CV was similar in young and elderly subjects.

differ over time among young and elderly subjects (95% CI for the difference: −2.9, 5.7). The CV ranged from 6.9% to 26.5% and from 8.6% to 32.9% in young and elderly subjects, respectively. We observed an eight fold interindividual variation in the ratio with a mean CV of 48.1% ± 2.9% and 42.7% ± 3.6% in young and elderly subjects, respectively.

Influence of gender, age, and smoking status on the PAX/CAF ratio

No effect of gender on the PAX/CAF ratio was observed either in young or in elderly subjects (95% CI for the differences: −0.26, 0.28, and −0.12, 0.20, respectively). The PAF/CAF ratio was significantly higher among young smokers than in young non-smokers (0.78 ± 0.19 *vs* 0.41 ± 0.19, $P = 0.004$). As shown in Table 1, a decrease in the PAX/CAF ratio was observed in older subjects compared with young subjects (0.52 ± 0.25 *vs* 0.34 ± 0.14, 95% CI for the difference, 0.004, 0.32). However, this difference was not age-related when nonsmokers were compared separately (0.41 ± 0.19 *vs* 0.32 ± 0.14 in 11 young and 13 elderly non smokers, respectively, 95% CI for the difference, −0.003, 0.21).

Discussion

The intraindividual CV of the ratio over time was comparable in young and elderly (range 6.9% to 26.5%, and 8.6–32.9%, respectively) but lower than those of previous reports using urinary caffeine metabolic ratios in young subjects (4.5% to 49.3%) [9] and that of the dextromethorphan metabolic ratio (up to 136%) used as a probe for CYP2D6 [10]. Interindividual variability in the PAX/CAF ratio was similar in our young and elderly healthy volunteers. A 70-fold variability has been found in caffeine urinary metabolic ratios [12] from a randomly selected Caucasian population. However in healthy non-smokers only a 4-fold variability was observed [8]. A 16-fold interindividual variability in CYP1A2 activity has been observed in the Chinese population [11]. These results reflect the high variability of CYP1A2 activity in humans, which is dependent on environmental covariates [13] and possibly additional genetic factors [11, 14].

The PAX/CAF ratio was reduced in elderly compared with young volunteers (Table 2) confirming previous *in vivo* [15, 16] and *in vitro* studies [17]. However the decrease in the plasma PAX/CAF ratio was not significant when non smokers were compared. This is in agreement with a study of 786 Caucasian subjects in which age was found not to influence CYP1A2 activity [13].

In conclusion, the PAX/CAF ratio used as a probe for CYP1A2 activity, was found to be related to the smoking status of subjects. However it did not vary over time regardless of age and smoking status.

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