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Effects of caffeine intake on the pharmacokinetics of melatonin, a probe drug for CYP1A2 activity

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Aims The aim of this study was to assess the influence of concomitant caffeine intake on the pharmacokinetics of oral melatonin**,** a probe drug for CYP1A2 activity.

Methods Twelve healthy subjects, six smokers and six nonsmokers, were given melatonin (6 mg) either alone or in combination with caffeine (3 × 200 mg). Blood samples for the analysis of melatonin or caffeine and paraxanthine were taken from 1 h before until 6 h after intake of melatonin. Subjects were genotyped with respect to the *CYP1A2 *1F* (*C734A*) polymorphism.

Results When caffeine was coadministered the C_{max} and AUC of melatonin were increased on average by 142% ($P = 0.001$, confidence interval on the difference 44, 80%) and 120% ($P < 0.001$, confidence interval on the difference 63, 178%), respectively. The inhibitory effect of caffeine was more pronounced in nonsmokers and in individuals with the **1F/*1F* genotype.

Conclusion The results of this study revealed a pronounced effect of caffeine on the bioavailability of orally given melatonin, most probably due to inhibition of CYP1A2 activity.

Keywords: caffeine, CYP1A2, melatonin

Introduction

Besides its role in drug metabolism, CYP1A2 has gained attention for its capability to activate procarcinogenes like aromatic- and heterocyclic amines [1, 2]. CYP1A2 activity varies widely between individuals, mostly due to the influence of environmental factors [3–6]. The only polymorphisms of functional relevance described so far have been found in intron 1 and the 5′ flanking region of the *CYP1A2* gene [7, 8]. Although a recent twin study has provided evidence for a contribution of genetic factors to the catalytic activity of CYP1A2 [9], a clear association between allelic variation and enzyme function is currently lacking, and thus phenotyping has been the only way of assessing CYP1A2 activity.

The metabolism of caffeine is more than 90% dependent on CYP1A2 activity [10], and thus caffeine clearance is considered as the 'golden standard' for assessment of CYP1A2 activity [11]. However, the results from caffeine phenotyping can be confounded by several factors [12]. Recently it has become evident that the pituitary hormone melatonin is quite a selective substrate for CYP1A2 and thus might be used as an alternative probe for this enzyme [13, 14].

The present study in healthy volunteers was performed to investigate the effect of caffeine on the pharmacokinetics of orally administered melatonin with special reference to smoking status and the *CYP1A2*1F (C734A)* polymorphism.

Methods

Subjects

Twelve healthy Finnish subjects (five females), aged between 18 and 40 years participated in the study. Six were smokers (at least 20 cigarettes/day) but none of the 12 subjects used any concomitant medications, including oral contraceptives. All volunteers gave their written informed consent prior to the study. The study protocol was approved by the first ethics committee of the hospital district of Varsinais-Suomi.

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Study procedure

Consumption of methylxanthine containing food and beverages was restricted for 48 h before and during each study day. Subjects were not allowed to drink alcohol or use any prescribed or nonprescribed drugs or herbal products for 2 days before and during the study. Subjects in the smoking group were required to document the number of cigarettes smoked over the last 2 days before and during the study days. The subjects were not allowed to consume charcoal grilled food or cruciferous vegetables on the study days and for 48 h preceding each study day. The investigation was conducted as an open, balanced, randomized, cross-over study with all subjects receiving the two alternative treatments (melatonin only or melatonin + caffeine) in random order. In period A of the study, subjects received a single oral dose of 6 mg melatonin (2 × 3 mg tablets, Yliopiston apteekki, Helsinki, Finland) at 08.30 h and venous blood samples (10 ml) were taken 1 and 0.5 h before, immediately before intake and 0.5, 1, 1.5, 2, 2.5, 3, 4, and 6 h after intake. In period B, 200 mg caffeine (2 × 100 mg tablets, Vitabalans, Hämeenlinna, Finland) was given 1 h before intake of 6 mg melatonin (at 08.30 h) and at 1 and 3 h afterwards. Blood was sampled at the same time points as in period A. There was a 1-week washout between the two periods. For the determination of genotype, a 10 ml whole blood sample was collected in an EDTA tube for the preparation of genomic DNA.

Caffeine assay

The concentrations of caffeine and paraxanthine in serum were analysed by a recently published HPLC method with UV-detection [15]. The interassay coefficients of variation from the analysis of control samples at 1 and 10 μM in the case of caffeine and 10 and 60 μM in case of paraxanthine were always less than 5% for both drugs. The limit of determination was 1 μM for both caffeine and paraxanthine.

Melatonin assay

Melatonin was measured by a radioimmunoassay (RIA) adapted from the method described by English *et al.* [16]. The limit of determination was less than 2 pg ml^{-1} and the interassay coefficient of variation (CV) was 16.3% at low concentrations (less than 20 pg ml^{-1}). To reach the linear range of the assay (2–500 pg ml^{-1}) samples were diluted 20 to 100 times. There was no crossreaction with either caffeine and metabolites or melatonin metabolites.

All samples were analysed in duplicate and reported as means.

Genotyping

Extracted DNA was subjected to a 5′–nuclease allele-specific assay (Taqman®) test specific for the C→A polymorphism in intron 1 as described by Nordmark *et al.* [17].

Data analysis

All pharmacokinetic parameters (area under the concentration-time curve, AUC (μg l^{-1} h), oral melatonin clearance, CL (l h^{-1} kg^{-1}), elimination rate constant, k_{el} (h^{-1})) were calculated by noncompartmental analyses using the Top-Fit pharmacokinetic data analysis software (version 2.0, Gustav Fischer Verlag, Stuttgart, Germany). The maximum concentrations (C_{max}) and the time to reach C_{max} (t_{max}) were taken directly from the concentration *vs* time data.

To compare the means of the two treatment regimens, melatonin only and melatonin + caffeine, data were log transformed and a paired *t*-test was applied.

The relationship between the difference in the pharmacokinetic parameters for melatonin between period A and B and the caffeine and paraxanthine concentrations was tested by the parametric Pearson correlation test.

P values less than 0.05 were considered statistically significant. All statistical analyses were performed with SPSS software (version 9.0, SPSS Inc., Chicago, USA).

Results

When caffeine was coadministered the C_{max} of melatonin was increased (Figure 1, Table 1) on average by 137% ($P = 0.001$, 95% confidence interval, CI, 44.3, 230%)

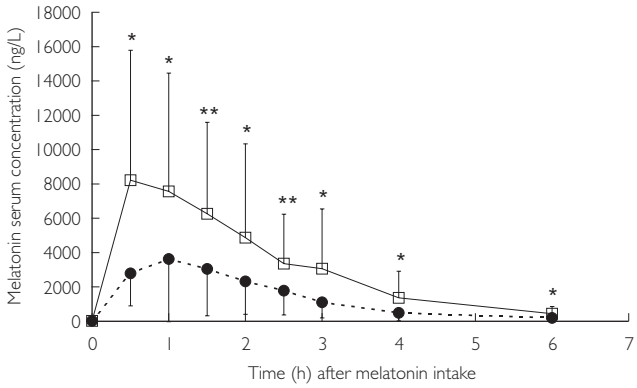

Figure 1 Melatonin serum concentration after oral administration of melatonin alone (closed circles, dotted line) or together with caffeine (3 × 200 mg) given 1 h before and 1 and 3 h after melatonin intake (open squares, solid line). Melatonin serum concentrations were significantly (*$P < 0.05$; **$P < 0.01$) higher at all time points between 0.5 and 6 h after melatonin intake when caffeine was coadministered.

Table 1 Melatonin pharmacokinetic parameters (mean and 95% confidence interval, 95% CI), in six healthy smoking and six healthy nonsmoking subjects derived after oral administration of 6 mg melatonin alone and after coadministration with caffeine (3 × 200 mg p.o).

	Melatonin only			*Melatonin + caffeine*		
	C_{max}[a] *(ng ml^{-1})* *(95% CI)*	*CL*[b] *(l h^{-1} kg^{-1})* *(95% CI)*	$t_{1/2}$[c] (h) (95% CI)	C_{max} *(% change)* *(95% CI)*	*CL (% change)* *(95% CI)*	$t_{1/2}$ *(% change)* *(95% CI)*
All	4.48	2.46	1.77	+ 137%**	−46.9% **	+ 6.50
(*n* = 12)	(2.20, 6.77)	(0.89, 4.04)	(1.12, 2.42)	(44, 230)	(−32.7, −61.0)	(−20.4, 33.4)
Smoking status						
Nonsmoker	5.61	1.48	1.11	+ 176.6%	−56.2%*	+ 2.52%
(*n* = 6)	(0.97, 10.2)	(0.76, 2.19)	(0.86, 1.35)	(−23.2, 376.4)	(32.4, 79.9)	(−22.1, 27.2)
Smoker	3.36	3.45	2.43	+ 97.2%*	−37.5%*	+ 10.5%
(*n* = 6)	(0.82, 5.89)	(0.02, 6.89)	(1.28, 3.58)	(11.5, 183)	(17.3, 57.8)	(−50.2, 71.2)

[a]maximum concentration, [b]total clearance, [c]apparent half-life, *$P < 0.05$, **$P < 0.01$.

and AUC by 120% ($P < 0.001$; 95% CI 62.7, 178%). Accordingly, oral CL was decreased, on average by 47% ($P = 0.02$, 95% CI −33, −61%). The apparent $t_{1/2}$ of melatonin was not affected by caffeine (Table 1). The differences in C_{max} and AUC between the two treatment periods were significantly correlated with caffeine concentration 2 h after intake of the first caffeine dose ($r = 0.696$; $P = 0.012$ and $r = 0.659$, $P = 0.02$, respectively) and with the last measured caffeine concentration ($r = 0.717$; $P = 0.009$ and $r = 0.861$, $P < 0.001$, respectively). No correlation was found with the paraxanthine concentrations.

Smokers had a lower C_{max} (3.36 ng ml^{-1}, 95% CI 0.82, 5.89) and AUC (5.5 μg l^{-1} h, 95% CI 2.13, 8.62 μg l^{-1} h) compared with nonsmokers (C_{max} = 5.61 ng ml^{-1} 95% CI 0.97, 10.2; AUC = 9.65 μg l^{-1} h, 95% CI 4.52, 18.55 μg l^{-1} h) when melatonin was given alone. As shown in Table 1, the inhibition of melatonin metabolism was in general more pronounced in nonsmokers (mean increase in C_{max} +197% and decrease in CL −56%, respectively) than in smokers (mean increase in C_{max} + 97% and decrease in CL −38%, respectively).

Our sample comprised seven subjects homozygous for the **1F* allele (**1F/*1F*), four heterozygous (**1F/*1A*) and one homozygous for the **1A* allele (**1A/*1A*). The nonsmoking subject carrying two **1A* alleles had by far the highest C_{max} of 14.3 ng ml^{-1} and AUC of 23.9 μg l^{-1} h and lowest oral CL of 0.63 l h^{-1} kg^{-1}. The mean increase in C_{max} was higher in subjects with the **1F/*1F* genotype (202%) compared with subjects carrying **1F/*1A* alleles (36%).

Discussion

The most prominent effect of caffeine on melatonin disposition was on the C_{max}, which was on average more than doubled. However, apparent half-life was not affected, suggesting an effect on the first-pass metabolism of melatonin leading to a higher oral bioavailability, the magnitude of which is similar to the previously reported effect of fluvoxamine, a known CYP1A2 inhibitor [18]. The caffeine dose of 200 mg corresponds to one large cup or two small cups of coffee and is comparable with that ingested at breakfast in many western countries [19].

The results of this study support concerns on concomitant intake of CYP1A2 substrates like theophylline, clozapine or olanzapine with caffeine. Moreover, if melatonin is used as a CYP1A2 probe concomitant caffeine intake is a confounding factor and needs to be avoided.

In summary, caffeine was found to increase the oral bioavailability of melatonin probably due to an inhibition of the CYP1A2 catalysed first-pass metabolism of melatonin. The effect was more pronounced in nonsmokers and subjects with the **1F/*1F* genotype.

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