Effect of quinolones on caffeine disposition

Six healthy volunteers received a single caffeine dose after pretreatment with norfloxacin, pipemidic acid, or placebo in a crossover, randomized, single-blind clinical trial. Quinolones altered the pharmacokinetics of caffeine, with a significant increase in the AUCs and a decrease in plasma clearance. The elimination half-life increased significantly with pipemidic acid. The apparent volume of distribution, mean renal clearance, and time to reach maximum caffeine concentrations remained unaltered. There was a decline in caffeine metabolite levels in the 24-hour urine samples for both quinolone treatments, suggesting that pipemidic acid and, to a lesser degree, norfloxacin inhibit metabolism of the *N*-demethylation pathways of caffeine. The practical consequence of this observation could be caffeine accumulation during repeated intake of coffee. In two additional healthy volunteers under a controlled multiple-dose regimen of caffeine ingestion, administration of pipemidic acid for 2 days caused a fourfold increase in the plasma concentrations of caffeine. (CLIN PHARMACOL THER 1989;45:234-40.)

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The primary pathways of caffeine metabolism are *N*-demethylations,[1] which take place in the hepatic microsomal oxidative system (cytochrome P-450) mediated by isozymes, also related to the biotransformation of polycyclic aromatic hydrocarbons.[2] Factors such as smoking habit,[3] pregnancy,[4,5] genetic inheritance,[6] liver disorders,[7] and interaction with other drugs like cimetidine,[8,9] disulfiram,[10] oral contraceptives,[11,12] and mexiletine[13] can moderately affect metabolic clearance of caffeine. Intense metabolic inhibition of caffeine *N*-demethylation by idrocilamide,[14] furafylline,[15] and methoxsalen[16] has been detected in human beings. Studies using animal models[17-19] confirmed these interactions and demonstrated that the phenomenon is shared by other compounds. Recent findings have shown that antibiotics of the quinolone group can affect the clearance of substances with a caffeine-like structure such as theophylline.[20-24] Side effects probably caused by caffeine accumulation have been observed in patients treated with enoxacin[25,26] and pipemidic acid.[27] Alterations in caffeine disposition as a result of treatment with enoxacin and ciprofloxacin have also been described.[28,29] To study how the quinolones alter caffeine disposition, a clinical trial has been carried out to evaluate the variations of the pharmacokinetic parameters and the metabolic profile of caffeine caused by treatment with pipemidic acid or norfloxacin. Caffeine accumulation after multiple doses in the presence of concomitant treatment with multiple doses of pipemidic acid was also investigated.

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SUBJECTS AND METHODS

Subjects. Eight healthy male volunteers (age range 20 to 37 years) were selected after evaluation of their clinical history, physical examination, ECG, and complete blood and urine analyses. Seven subjects were regular smokers (10 to 40 cigarettes per day) and volunteer No. 8 was a nonsmoker. The subjects had not taken drugs during the 4 weeks before the trial. They were informed of extent, nature, and objectives of the study and gave written consent. All the studies were approved by the Hospital del Mar Ethical Committee and authorized by the Dirección General de Farmacia y Productos Sanitarios (Nos. 87/260). Drugs were administered orally with 120 ml water after a 10-hour fast. The volunteers were requested to abstain from the

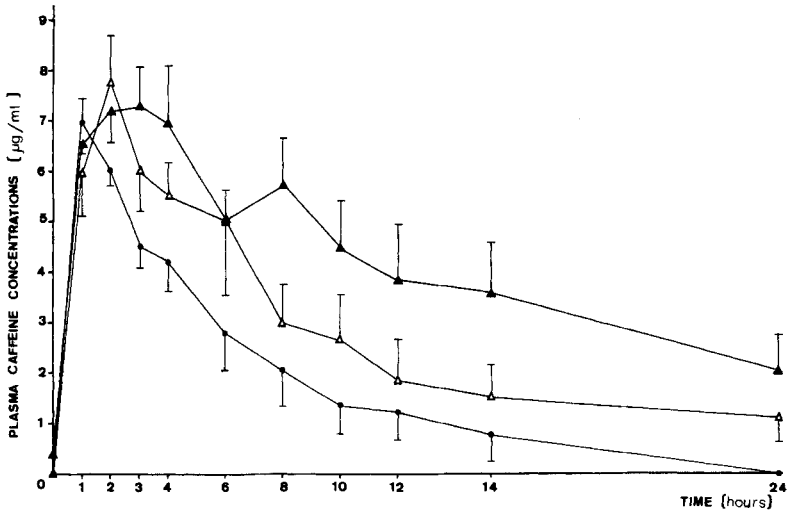

Fig. 1. Caffeine plasma concentrations for six volunteers (mean ± SE) after administration of a single dose of caffeine and treatment with pipemidic acid *(black triangles)*, norfloxacin *(white triangles)*, or placebo *(black circles)*.

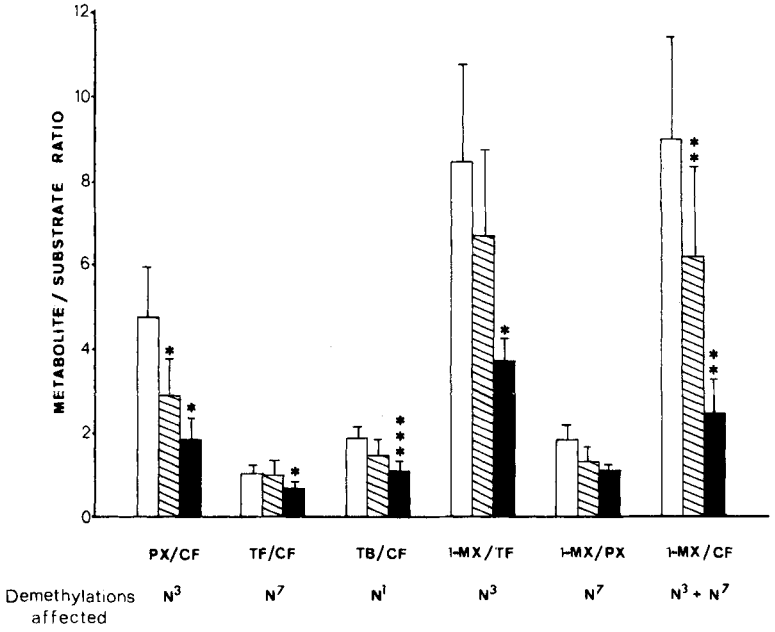

Fig. 2. Concentrations ratios (mean ± SE) of *N*-demethylated caffeine *(CF)* metabolites in 24-hour urine samples after administration of a single dose of caffeine and treatment with pipemidic acid *(black bars)*, norfloxacin *(stripped bars)*, or placebo *(white bars)*. The *N*-demethylations affected are indicated at the bottom of the figure. Ratios are indicated as follows: paraxanthine/caffeine *(PX/CF)*, theophylline/caffeine *(TF/CF)*, theobromine/caffeine *(TB/CF)*, 1-methylxanthine/theophylline *(1-MX/TF)*, 1-methylxanthine/paraxanthine *(1-MX/PX)*, and 1-methylxanthine/caffeine *(1-MX/CF)*. (* $0.02 < p < 0.05$ vs placebo; ** $0.005 < p < 0.02$ vs placebo; *** $p < 0.005$ vs placebo.)

Table I. Pharmacokinetic parameters for caffeine after single dose and during treatment with pipemidic acid, norfloxacin, or placebo

Volunteer			*Placebo*		
No.	*Age (yr)*	*Weight (kg)*	C_{max} *(μg/ml)*	t_{max} *(hr)*	$t_{l/2}$ *(hr)*
1	23	74.5	6.5	1.0	3.53
2	23	61.7	8.11	1.0	3.71
3	22	59.4	5.87	2.0	1.02
4	19	60.5	8.17	1.0	13.47
5	21	60.5	6.36	1.0	2.77
6	22	69.5	7.35	2.0	3.40
$\bar{X}$ ± SE	21.6 ± 0.6	64.3 ± 2.5	7.06 ± 0.39	1.3 ± 0.2	4.65 ± 1.81
p Value					

Norfloxacin							
C_{max} *(μg/ml)*	t_{max} *(hr)*	$t_{l/2}$ *(hr)*	$AUC_{(0\text{-}24)}$ *(mg · hr/L)*	$AUC_{0\text{-}\infty}$ *(mg · hr/L)*	V_{area} *(L/kg)*	*CL (L/hr)*	CL_R* *(L/hr)*
4.66	1.0	5.41	45.88	45.88	0.80	7.63	0.041
6.53	1.0	4.11	50.08	50.08	0.67	6.99	0.093
7.86	1.0	2.03	32.18	32.18	0.54	10.88	—
9.92	2.0	12.33	116.22	161.06	0.64	2.17	0.260
8.27	1.0	2.68	48.93	48.93	0.46	7.15	0.134
10.52	2.0	7.63	108.30	124.59	0.45	2.81	0.070
7.96 ± 0.88	1.3 ± 0.2	5.70 ± 1.56	66.93 ± 14.60	77.12 ± 21.46	0.59 ± 0.05	6.27 ± 1.33	0.120 ± 0.038
NS	NS	NS	<0.05	NS	NS	<0.01	NS

NS, not significant differences vs placebo.
*n = 5 (loss of urine volume of volunteer 3).

consumption of alcohol and drinks and foods that contained methylxanthines for 48 hours before the onset of each phase of treatment.

Single caffeine dose pharmacokinetic study. Six volunteers (Nos. 1 to 6) were subjected to a Latin square design with single-blind administration according to previous random assignment. There were three phases of treatment, each lasting 2 days and separated by a 7-day drug-free interval. During the first day of each phase, volunteers received one of the following treatments: pipemidic acid, 800 mg b.i.d., norfloxacin, 800 mg b.i.d., or placebo b.i.d. On the second day of each phase each volunteer was given a single dose of caffeine (350 mg) together with the first daily dose of the assigned drug, the antecubital vein was catheterized, and a 10 ml blood sample was drawn before caffeine administration (t = 0) and 1, 2, 3, 4, 6, 8, 10, 12, 14, and 24 hours after administration. Twenty-four-hour urine samples were also collected. Plasma and urine samples were stored at −20° C until analysis. Vital signs were recorded immediately before blood extractions and self-reported side effects were assessed at 0, 14, and 24 hours.

Multiple caffeine dose study. Volunteers 7 and 8 were submitted to controlled caffeine intake for 48 hours before pipemidic acid administration (days 1 and 2 of this protocol), which was continued until day 5. Doses were 100 mg q.i.d. for volunteer 7 and 100 mg t.i.d. for volunteer 8. On days 3 and 4 they also received 400 mg b.i.d. pipemidic acid. Blood samples were obtained throughout the study and handled as described above.

Analysis of biologic samples. Plasma samples were taken at room temperature and subjected to an ultrasound bath for 30 minutes. More than 250 μl plasma was dispensed in 500 μl of methanolic solution containing ethyltheophylline (10 μg/ml) as internal standard. The sample was vortexed for 20 seconds and then centrifuged at 3000 rpm for 15 minutes. The methanolic supernatant was collected and 20 μl injected into an HPLC system. The HPLC mobile phase was a mixture of 2% tetrahydrofurane in 10 mmol/L sodium sulfate adjusted to pH 6.5 with phosphoric acid/acetonitrile/methanol (80:10:10). Spherisorb ODS-1 column (Phase Separation Ltd., Queenserry, U.K.), 20 by 0.46 cm, particle size 10 μm, and detection wavelength of 280 nm were used. The assay sensitivity for caffeine was 0.3 μg/ml. The interassay coefficient of variation

Placebo				
$AUC_{(0\text{-}24)}$ (mg · hr/L)	$AUC_{0\to\infty}$ (mg · hr/L)	V_{area} (L/kg)	CL (L/hr)	CL_R* (L/hr)
30.91	30.91	0.78	11.32	0.113
42.71	42.71	0.71	8.19	0.175
20.09	20.09	0.43	17.42	—
86.67	86.67	1.29	4.03	0.113
34.24	34.24	0.67	10.22	0.093
49.87	49.87	0.49	7.01	0.063
44.08 ± 9.48	44.08 ± 9.48	0.73 ± 0.124	9.70 ± 1.86	0.111 ± 0.018

Pipemidic acid							
C_{max} (μg/ml)	t_{max} (hr)	$t_{1/2}$ (hr)	$AUC_{(0\text{-}24)}$ (mg · hr/L)	$AUC_{0\to\infty}$ (mg · hr/L)	V_{area} (L/kg)	CL (L/hr)	CL_R* (L/hr)
6.38	1.0	7.16	68.30	76.15	0.64	4.6	0.076
7.72	2.0	10.32	90.75	116.66	0.72	3.00	0.290
7.41	1.0	3.86	45.52	45.52	0.72	7.68	—
11.89	4.0	19.73	141.91	274.58	0.60	1.27	0.157
7.13	3.0	9.08	82.95	101.82	0.74	3.43	0.038
8.24	3.0	19.58	150.03	254.29	0.56	1.38	0.043
8.13 ± 0.79	2.3 ± 0.5	11.62 ± 2.69	96.58 ± 16.87	144.84 ± 39.17	0.66 ± 0.03	3.56 ± 0.97	0.121 ± 0.05
NS	NS	<0.02	<0.005	<0.05	NS	<0.005	NS

was 7% at 1 μg/ml and 5.4% at 10 μg/ml (n = 8). Intrassay coefficient of variation was 4.6% and 3.2% (n = 8) at the same concentrations.

Caffeine and its metabolites were analyzed in urine samples according to the method developed by Grant et al.[30] with the following modifications. More than 0.5 ml urine was dispensed in 0.2 ml of methanolic solution containing 8-chlorotheophylline as internal standard (10 μg/ml). Six milliliters of chloroform/isopropanol (85:15) was added and the mixture was shaken for 30 seconds and centrifuged for 5 minutes at 2500 rpm. The organic phase was transferred and evaporated; the residue was reconstituted in 400 μl mobile phase and 20μl was injected into an HPLC system. Assay sensitivity was 0.1 μg/ml for caffeine and metabolites; the corresponding interassay coefficients of variation were 5.5% at 1 μg/ml and 3.1% at 10 μg/ml (n = 10). Intraassay coefficients of variation were 4.1% and 2% (n = 10).

Data analysis. Pharmacokinetic parameters are described in Table I. Experimental values are the maximum plasma concentrations (C_{max}), time to reach this value (t_{max}), and $AUC_{0\to24}$.

Calculations were carried out to determine the best least-squares fit of the individual curves to a monocompartmental model. The resulting plasma elimination half-life ($t_{1/2}$), apparent volume of distribution (V_{area}), and area under the curve from zero to infinity ($AUC_{0\to\infty}$) are also reported together with the total plasma clearance (CL) and mean renal clearance (CL_R), calculated as total caffeine excreted in 24-hour urine divided by $AUC_{0\to24}$. Complete absorption was assumed.[31]

The paired Student t test was used for each volunteer (single dose study) to evaluate the effects of pipemidic acid and norfloxacin on pharmacokinetic parameters compared with placebo values.

RESULTS

Quinolone effects on pharmacokinetics of caffeine. Fig. 1 shows the plasma caffeine concentrations of volunteers in the caffeine single-dose study and Table I presents the individual pharmacokinetic parameters. C_{max} was usually attained 1 to 3 hours after caffeine administration. Plasma caffeine levels were significantly greater in the pipemidic acid treatment group than in the placebo group at times from 3 hours on. At 8 and 24 hours, caffeine levels of 5.7 ± 2.33

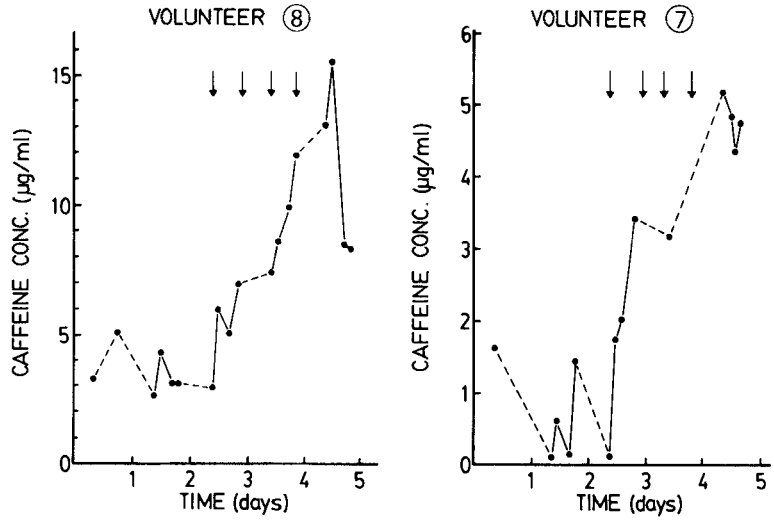

Fig. 3. Caffeine plasma concentrations in volunteers 8 (nonsmoker) and 7 (smoker) after several caffeine doses per day. *Arrows* indicate pipemidic acid administration.

μg/ml and 2.0 ± 1.78 μg/ml were reached with pipemidic acid compared, respectively, with 2.2 ± 1.82 μg/ml and undetectable in the placebo group. Average levels were always higher for the norfloxacin group than for the placebo group, although there were significant differences only between 3 and 4 hours after caffeine administration.

The elimination $t_{1/2}$ of caffeine was 248% greater ($p < 0.02$) in the pipemidic acid group. Plasma clearance decreased for both the pipemidic acid ($p < 0.005$) and norfloxacin ($p < 0.01$) groups. $AUC_{0\rightarrow 24}$ increased for both pipemidic acid ($p < 0.005$) and norfloxacin ($p < 0.05$). Apparent V_{area}, mean CL_R, C_{max}, and t_{max} did not vary significantly.

Ratios of the urinary concentrations of caffeine metabolites to their respective substrates along the metabolic pathways (Fig. 2) showed that paraxanthine/caffeine, theophylline/caffeine, theobromine/caffeine, 1-methylxanthine/caffeine, and 1-methylxanthine/theophylline decreased with pipemidic acid administration. After norfloxacin administration, only paraxanthine/caffeine and 1-methylxanthine/caffeine showed significant differences with regard to the placebo. The ratio 1-methylxanthine/paraxanthine did not vary significantly as a result either of pipemidic acid or norfloxacin pretreatments. However, independent of the statistically significant differences, all mean values of the ratios were always lower after the quinolone than after placebo treatments.

Quinolone effects on caffeine steady-state plasma caffeine levels. After repeated administration of caffeine (Fig. 3), plasma caffeine concentrations in volunteer 8 stabilized at about 3 to 5 μg/ml and then increased after pipemidic acid intake, reaching levels of about 15 μg/ml. The plasma levels of volunteer 7 stabilized at about 0.1 to 1.5 μg/ml and increased to 5 μg/ml after pipemidic acid treatment. In both cases there was a fourfold increase in plasma concentrations after 2 days of pipemidic acid treatment. No adverse effects were observed in either volunteer.

DISCUSSION

The results show that caffeine elimination is inhibited by both pipemidic acid and norfloxacin. The differences in pharmacokinetic parameters suggest that the alterations in caffeine clearance induced by pipemidic acid and norfloxacin are quantitatively different, pipemidic acid inhibiting caffeine elimination more than norfloxacin. These differences are not surprising, because recent studies involving other quinolones (i.e., enoxacin and ciprofloxacin[28,29]) have also shown a difference in the degree of effects on plasma caffeine clearance.

The absence of significant differences with respect to the placebo in both the apparent V_{area} and the mean CL_R suggests that the alteration in caffeine disposition is not related to changes in the distribution or renal elimination of the unaltered drug. Caffeine absorption was not affected by pretreatment with the quinolones, because neither t_{max} nor C_{max} varied for either drug. This fact is in agreement with the assumption of complete bioavailability of caffeine after oral administration,[31,32]

These findings suggest altered metabolic elimination

as the most probable mode of interaction. Primary metabolic degradation of caffeine takes place in the hepatic mixed-function oxidase system (cytochrome P-450, forms c and d) by *N*-demethylation, N^3-demethylation being the principal route of biotransformation.[2] This metabolic pathway seems to be the one most affected by quinolone treatment, because of the relative reduction in the paraxanthine/caffeine ratio in 24-hour urine samples (60% and 39% for the pipemidic acid and norfloxacin groups, respectively). The important decrease in the 1-methylxanthine/theophylline ratio is also related to inhibition of the N^3-demethylation. The differences in the theobromine/caffeine, theophylline/caffeine, and 1-methylxanthine/paraxanthine ratios suggest that pipemidic acid inhibits not only N^3-demethylation but also to some extent N^7- and N^1-demethylation pathways. Obviously, those metabolites subjected to consecutive metabolic pathways potentially affected show a significant inhibition (e.g., 1-methylxanthine/caffeine; Fig. 2).

The caffeine multiple-dose study confirmed the alterations in caffeine disposition, because plasma concentrations increased after pipemidic acid administration. Rapid accumulation was induced even under conditions of normal caffeine intake. The plasma levels of volunteers 7 and 8, like those in the single-dose study, did not produce adverse effects. This is probably due in part to the fact that all volunteers except number 8 were regular cigarettes smokers. It is known that smoking produces enzymatic induction of caffeine biotransformation,[33] which blunts the effects of quinolone inhibition. Volunteer 8 (nonsmoker) probably would have attained caffeine plasma concentrations sufficient to produce symptoms of caffeine intoxication if the regimen of pipemidic acid administration had continued. Those situations in which alteration in caffeine disposition was caused by other enzymatic inhibitors (oral contraceptives, cimetidine, disulfiram, methoxalen, etc.) should be given special attention when treatment with antibiotics of the quinolone group is considered.

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