Assessment of the Time Course of Drugs With Inhibitory Effects on Hepatic Metabolic Activity Using Successive Salivary Caffeine Tests

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In clinical practice is very important to know the time course of the inhibitory effects of drugs to avoid side effects when several agents are taken concomitantly. We attempted to validate the effectiveness of successive salivary caffeine tests establishing the time for cimetidine to inhibit hepatic metabolism. The time of cimetidine's inhibitory effect as reported in other studies was chosen as the reference. In this open-label, prospective longitudinal, 16-day study, five healthy volunteers were treated with cimetidine 1 g/day for 5 days. After the intake of caffeine 300 mg, salivary caffeine tests were carried out on days -1 (control value), 1, 4, 8, 12, and 16. The mean systemic caffeine clearance was decreased after 24 hours of cimetidine. The drug's maximum inhibitory effect was reached after 5 days of administration, returning to previous values progressively 1–7 days after discontinuing cimetidine. No change occurred in the apparent volume of distribution. The time course of cimetidine's inhibitory effect was similar to that described with other methodologies. Although this was a pilot trial and the results have to be confirmed, it seems that successive salivary caffeine measurements could be a safe, reliable, noninvasive test for exploring the time course of the inhibitory effects of drugs on hepatic metabolism.
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The liver is the main site of metabolism for most drugs, so it is important to know the time of an agent's inhibitory effect on hepatic metabolism. The most frequently reported clinical manifestation of inhibition of hepatic metabolism is toxicity of the inhibited drug, ranging from mild clinical problems to severe adverse reactions and death. These effects can usually be avoided if inhibition is anticipated. Therefore, when an inhibitor is administered to a patient who is taking other drugs, it is necessary to know the time course of inhibition, its onset, time to maximum effect, and duration.[1]

Antipyrine has been widely used to appraise the activity of the hepatic drug-metabolizing enzymes,[2] but it must not be used to assess the time of inhibition because it is itself a mild enzyme-inducer in humans, and repeated measurements of its clearance can stimulate its own metabolism.[3] D-glucaric acid and 6-β-hydroxycortisol urinary excretion also have been given for this purpose, because these agents do not require administration of any exogenous substance.[4]

Caffeine has recently received increased attention as a compound that may be useful for quantitative measurement of liver function, as well as providing a suitable and safe test for exploring the potential of drugs to induce or inhibit hepatic enzyme metabolism.[5, 6] Reasons for considering caffeine are its complete absorption from the gastrointestinal tract; its rapid diffusion in total body water; it is only slightly bound by serum proteins; it is

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exclusively metabolized by the liver (the main enzymes involved in its metabolism are CYP1A2, NAT2, and xanthine oxidase)[7]; it has a low hepatic extraction ratio that is independent of liver flow (only 1–3% of an administered dose is recovered unchanged in the urine); and its lack of adverse effects at usual doses.[8,9]

Studies confirmed the excellent correlation of caffeine elimination in saliva and plasma,[10,11] and thus it is possible to perform the caffeine elimination test in saliva to measure liver function.[12] Cimetidine has powerful inhibitory effects on hepatic metabolism.[13] Its time course (onset, time to maximum effect, termination of enzyme inhibition) were studied in detail by several authors using different methods.[14–19]

We evaluated the time course of the inhibitory effect of cimetidine in healthy volunteers with consecutive salivary caffeine tests, and compared our findings with results of those studies[14–19] to validate the effectiveness of the tests.

Materials and Methods

Subjects

The subjects were three men and two women ranging in age from 23–29 years (mean 26 yrs). The body weights ranged from 55–75 kg (mean 63 kg). All were nonsmokers and reported only casual alcohol consumption, and none were taking any other drugs. Both women were studied at the midcycle ovulatory phase.

The subjects were determined to be healthy on the basis of history, physical examination, and electrocardiogram. In addition, they were within the normal limits for plasma urea, electrolytes, creatinine, bilirubin, aspartate aminotransferase, alkaline phosphatase, total protein, albumin, and creatinine clearance, and complete blood count. Written informed consent was obtained from all subjects, and the study was approved by the research and ethics committees of our hospital.

Protocol

All subjects were instructed to abstain from methylxanthine consumption (coffee, tea, caffeinated soft drinks, chocolate) for 3 days before and throughout the 16-day study. They also were requested to refrain from consuming any form of alcoholic beverage and smoking for the duration of the study. Caffeine tests were performed on days -1 (control), 1, 4, 8, 12 ,and 16. Oral cimetidine 200 mg 3 times/day and 400 mg at night was given on days 0, 1, 2, 3, and 4 (Table 1). Compliance with the protocol was verified by tablet counts.

Table 1. Study Protocol

Study Day	Intervention
Pre-study	72-Hour methylxanthine-free diet (no tea, coffee, chocolate)
-1	Caffeine test (control value)
0	Cimetidine dose
1	Caffeine test
	Cimetidine dose
2 and 3	Cimetidine dose
4	Caffeine test
	Cimetidine dose
8	Caffeine test
12	Caffeine test
16	Caffeine test

On each day of the study the subjects were permitted their usual breakfast, excluding caffeine. A baseline sample of stimulated saliva (5 ml) was collected, and then each subject received a total of 300 mg of anhydrous caffeine diluted in milk. They were instructed to rinse their mouths thoroughly immediately after ingesting the caffeine. Saliva samples (5 ml mixed saliva) were collected in polyethylene vials by means of chewing a strip of paraffin at 3, 6, 9, 12, 18, and 24 hours after ingestion of caffeine and centrifuged at 2500 rpm for 5 minutes. Supernatant 2 ml was collected and stored at -50°C until assayed within 3 days of collection.

Data Analyses

The concentrations of caffeine were determined in duplicate by high-pressure liquid chromatography using a selective method that measured only caffeine without metabolites,[20] with intraassay and interassay coefficients of variation of 6% and 8%, respectively. The detection limit was 0.2 µg/ml and the detection range was linear up to 20 µg/ml.

Caffeine concentration-time curves were analyzed by assumption of a first-order one-compartment model. Saliva caffeine concentrations were plotted semilogarithmically as a function of time, and the slope of the descending curve (elimination rate constant, k_{el}) was calculated by log linear least squares regression analysis. The areas under the saliva concentration-time curves (AUCs) from zero to infinity were estimated by the trapezoidal method.[21] For pharmacokinetic analysis the following equations were used:

$$\text{Half-life} = 0.693/k_{el},$$

Total saliva clearance Cl = dose/AUCs, and

Table 2. Saliva Clearance (L/hr) and Half-Life (hrs) of Caffeine[a]

	Study Day											
	Basal		1		4		8		12		16	
	Cl	$T_{1/2}$	Cl	$T_{1/2}$	Cl	$T_{1/2}$	Cl	$T_{1/2}$	Cl	$T_{1/2}$	Cl	$T_{1/2}$
	3.8	5.7	4.1	6.9	2.2	10	1.2	11.5	3.6	6.8	4.2	5.8
	7.1	4.5	6.1	5.7	5.2	4.9	6.8	4.4	7.9	4	9.4	3.4
	3.6	6.9	2.6	7.2	3.2	6.9	2.7	7.1	4.6	5.7	2.9	7
	3.4	9.6	2	12.3	2.3	14.2	2.5	8.9	3.2	9	3.4	8.8
	5.9	5.2	7.3	3.7	5.2	4.9	9.1	3	4.7	5.9	4.2	5.8
Mean	4.7	6.3	4.4	7.1	3.6	8.2	4.4	6.9	4.8	6.2	4.8	6.1

Cl = saliva clearance; $T_{1/2}$ = half-life.

[a]There were no significant statistical differences.

Apparent volume of distribution $V_d = Cl/k_{el}$.

Pharmacokinetic data were calculated using the computer-based program NONLIN.[22]

The results are expressed as means. The time course of caffeine clearance was analyzed using repeated measures analysis of variance (ANOVA) with the SPSS statistical program. The Tukey posttest was used for multiple comparisons. A p value less than 0.05 was considered statistically significant.

Results

The pharmacokinetic parameters of caffeine before, during, and after treatment with cimetidine are shown in Table 2. Caffeine clearance decreased quickly after the intake of cimetidine, and its maximum inhibitory effect was attained while cimetidine was administered, although without statistical significance.

After the withdrawal of cimetidine, caffeine clearance returned to baseline values progressively along several days. No change in apparent volume of distribution was observed. None of the volunteers experienced any side effects.

Discussion

Inhibition of hepatic metabolism may be important when the inhibitor drug is started or stopped, or its dosage is changed. However, inhibitors usually can be given safely if clinicians anticipate and plan for the expected pharmacologic effects, readjusting the dosages of concomitant drugs, thus preventing toxicity. Therefore, in clinical practice it is important for clinicians to know the time course of the inhibitory effect of drugs.

The time course of the inhibitory effect of cimetidine is well known. It inhibits drug metabolism within the first day of administration,[14, 15] although its maximum inhibitory effect occurs several days later.[16, 17] After cimetidine treatment is stopped, its inhibitory effect dissipates progressively over several days, returning enzyme activities to pretreatment levels.[18, 19] In our study, the time course of cimetidine's inhibitory effect was similar to that described by others, beginning on the first day, maximum effect after several days, and progressive recovery of baseline values several days after drug withdrawal.

This study was only a pilot trial and had some limitations, especially the small number of patients, its relative short duration, and the limited number of measurements of caffeine carried out each day. However, our results suggest that successive salivary caffeine tests could be a simple, safe, useful, and suitable method for evaluating the time course of drugs with inhibitory effects on hepatic metabolic capacity. Further studies should be performed, modifying this protocol to include more patients, more measurements of caffeine each day, and a longer duration to confirm and validate these findings.

Urinary metabolite profiles of caffeine reflect systemic clearance,[23] so caffeine has been employed for several purposes: to determine acetylator status,[24, 25] to appraise the phenotype of some hepatic enzymes (CYP1A2 and NAT2),[26] to explore possible pharmacokinetic differences in subgroups of populations and in various clinical conditions,[27–29] and to investigate the influence of drugs on the activity of a specific enzyme of hepatic cytochrome P-450 family (P-4501A2).[30] Additional research should be conducted to determine if it is possible to use this methodology for assessing the time course of drugs with inhibitory effects on hepatic metabolism.

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