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Caffeine Elimination: A Test of Liver Function

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Summary. Fasting plasma caffeine concentration and various parameters of caffeine elimination from plasma obtained after a standardized oral dose of 140 mg caffeine have been compared in nine patients with liver cirrhosis, eight patients with non-cirrhotic liver disease and ten healthy volunteers with regard to their ability to discriminate between the different groups. Fasting plasma caffeine concentrations were significantly higher in cirrhotics (11.1 ± 10.5 μmol/l) than in healthy volunteers (1.5 ± 0.8 μmol/l). The respective values measured in patients with non-cirrhotic liver disease (3.1 ± 3.1 μmol/l) did not differ significantly from the controls. Plasma disappearance rate and clearance of caffeine were significantly decreased in cirrhotics ($0.11 \pm 0.02\ h^{-1}$; 1.0 ± 0.3 ml/min per kg) and in patients with non-cirrhotic liver disease ($0.18 \pm 0.04\ h^{-1}$; 2.2 ± 0.7 ml/min per kg) as compared to healthy volunteers ($0.23 \pm 0.04\ h^{-1}$; 3.1 ± 0.9 ml/min per kg). Plasma caffeine concentration determined 12 h after administration of the test dosage discriminated best between patients with cirrhosis (5.4 ± 1.6 μmol/l), patients with non-cirrhotic liver disease (2.0 ± 1.4 μmol/l) and healthy volunteers (0.8 ± 0.2 μmol/l). These results, the safety of the test compound and the simplicity of a single caffeine determination in plasma 12 h after a standardized dose of caffeine make this test attractive for evaluation of liver function.

Key words: Liver disease – Liver function – Caffeine elimination

In spite of the existence of a number of quantitative tests of liver function such as elimination of bromosulfophthalein [5], indocyanine green [8], bile acids [6], galactose [11], antipyrine [1] and aminopyrine [3], the use of quantitative tests of liver function has not gained wide acceptance. Most of these tests are not popular because they are time consuming, cumbersome and expensive or employ test substances which carry the risk of adverse effects. Therefore, a test substance is needed which has not been associated with adverse effects, can be easily measured in plasma, and is exclusively metabolized by the liver. Recently, it has been shown that caffeine comes close to fulfill these requirements [4, 10]. Caffeine is an inexpensive test substance which does not exhibit adverse effects at the dosages employed. It is rapidly and completely absorbed after oral intake and undergoes virtually complete metabolism by the liver [2]. A simple enzyme immunoassay for determination of caffeine plasma levels has become commercially available [9]. Desmond et al. [4] and Renner et al. [10] have demonstrated that caffeine plasma clearance is reduced in patients with liver disease and can be used as a quantitative test of hepatic microsomal function. Moreover, in coffee drinkers a significant relationship was found between the fasting caffeine concentration in plasma and the plasma clearance of caffeine [10]. It was suggested that, because of the ubiquitous consumption of caffeine, the fasting caffeine concentration might serve as a simple guide for the evaluation of the severity of liver disease [10]. Although this approach seems very attractive because of its simplicity, it may have the disadvantage of being disturbed by a number of uncontrolled variables such as the cumulative amount of caffeine consumed on the previous day and the time interval between the last caffeine intake and blood sampling. Therefore, the measurement of plasma caffeine may be more meaningful and may have a higher diagnostic value if it is

Abbreviations: Cl = clearance; ICG = indocyanine green; k = disappearance rate constant; V_d = volume of distribution

Table 1. Clinical and laboratory data of the patients studied ($\bar{x} \pm SD$; range in parenthesis)

Group	Number of patients (*n*)	Sex (m/f)	Age (years)	Serum bilirubin (mg/dl)	Aspartate aminotransferase (IU/l)	Alkaline phosphatase (IU/L)	Prothrombin time (%)	Serum albumin (g/dl)	Smokers (*n*)	Drug treatment (*n*)
Cirrhosis	9	8/1	57±10 (44–71)	1.4±0.7 (0.6–2.9)	37 ± 41 (4.8–123)	236±121 (123–482)	74± 21 (44–100)	3.9±0.8 (2.4–4.9)	3	5[a], 2[b], 1[c]
Non cirrhotic liver disease	8	7/1	46±12 (21–62)	2.5±2.1 (0.7–3.4)	74 ±101 (7.9–285)	234±168 (85–611)	91± 15 (56–100)	4.7±0.6 (3.9–5.5)	2	2[b]
Normal range				0.1–1.0	5 – 24	<190	70–100	3.5–5.0		

[a] = spironolactone
[b] = ranitidine
[c] = cimetidine

performed at a certain point of time after administration of a standardized dose of caffeine. The present study was designed to investigate which of the various parameters of caffeine elimination discriminates best between patients with liver disease and healthy controls.

Materials and Methods

Subjects

Seventeen patients with histologically documented liver disease and ten healthy controls were included in the study. The patients with liver disease were divided into two groups. The first group consisted of nine patients with cirrhosis (5 alcoholic, 4 post-hepatitic), the second group consisted of eight patients with non-cirrhotic liver disease (three patients with fatty liver, two with resolving viral hepatitis, one with chronic persistent hepatitis, one with chronic cholangitis and one with an early non-cirrhotic stage of primary biliary cirrhosis). In all patients the diagnosis was based on clinical, laboratory and histological findings. The clinical and laboratory data as well as the history of drug intake and smoking are given in Table 1.

Ten healthy subjects (five females, five males) aged 25–30 years were selected from the medical staff of the hospital. No signs of liver disease or of any other gastrointestinal disease were present. They were not taking any drugs including oral contraceptives. Five of them were smokers. All subjects gave informed consent before submission to the study.

Study Design

Following a 12-h overnight fast a blood sample was taken in the morning for determination of the fasting plasma caffeine concentration. Thereafter, all patients and healthy volunteers were kept on caffeine abstinence for a 4-day period. On the third day an indocyanine green (ICG) clearance test was performed. In the fasting state ICG (Hynson, Wescott and Dunning, Baltimore, MD, USA) was injected intravenously (0.5 mg/kg body weight) and 3 ml blood samples were collected at 3 min intervals up to 21 min. On the fourth day 140 mg caffeine were administered orally in a cup of specially prepared coffee (a kind gift of Dr. M.J. Arnaud from Nestlé Research Department, La Tour-de-Peilz, Switzerland). Thereafter, blood was sampled at 3-h intervals up to 12 h after ingestion of caffeine.

Analytical Methods

Caffeine. Caffeine concentration in plasma was determined by capillary gas chromatography and flame ionisation detection. To 0.5 ml plasma, antipyrine (Sigma Chemical Company, St. Louis, MO, USA) in a concentration of 10.6 µmol/l was added as internal standard. The sample was then alkalized with 0.5 ml 1 mol/l NaOH and extracted twice with five volumes of chloroform. After removal of the aqueous phase, chloroform was evaporated to dryness and the residue taken up into 1 ml 80% methanol. This phase was then cleaned up by extracting twice with 300 µl n-hexane. After removal of n-hexane, the aqueous methanol phase was evaporated and the residue taken up in 25 µl chloroform. 0.2–1.0 µl were injected onto a 25 m × 0.32 mm fused silica capillary OV-1701 column (CP Sil 19 CB, Chrompack, Middelburg, The Netherlands) using on column injection at 80° C and secondary injector cooling. After 1 min, the temperature was increased up to 200° C at a rate of 20° C/min. Detector temperature was kept at

Table 2. Fasting serum bile acids, indocyanine green (ICG) clearance, fasting plasma caffeine concentration and parameters of caffeine elimination after a standardized oral dose (140 mg) of caffeine in patients with liver disease and in healthy controls ($\bar{x} \pm SD$; range in parenthesis)

Group	Number of patients	Fasting serum bile acids	ICG clearance	Caffeine				
				Fasting plasma concentration	Plasma disappearance rate constant (k)	Volume of distribution	Plasma clearance	12 h concentration
	(*n*)	(μmol/l)	(ml/min per kg)	(μmol/l)	(h^{-1})	(ml/kg)	(ml/min per kg)	(μmol/l)
Cirrhosis	9	15.1 ± 11.5 (7.0 – 43)	4.2 ± 2.6 (1.4 – 9.5)	11.1 ± 10.5 (0.5 – 32.8)	0.11 ± 0.02 (0.06 – 0.15)	0.6 ± 0.2 (0.4 – 1.0)	1.0 ± 0.3 (0.7 – 1.7)	5.4 ± 1.6 (2.4 – 7.3)
Non cirrhotic liver disease	8	5.4 ± 1.6 (3.6 – 8.4)	6.3 ± 2.2 (4.2 – 10.3)	3.1 ± 3.1 (0.2 – 8.4)	0.18 ± 0.04 (0.11 – 0.21)	0.8 ± 0.3 (0.6 – 1.2)	2.2 ± 0.7 (1.0 – 3.3)	2.0 ± 1.4 (1.3 – 5.3)
Healthy volunteers	10	1.4 ± 0.8 (0.5 – 2.8)	10.1 ± 2.3 (6.9 – 13.9)	1.5 ± 0.8 (0.1 – 3.1)	0.23 ± 0.04 (0.17 – 0.29)	0.9 ± 0.3 (0.4 – 1.6)	3.1 ± 0.9 (1.9 – 5.0)	0.8 ± 0.2 (0.4 – 1.1)

280° C. The gas chromatograph used was a Carlo Erba, Fractovap 4160. Quantitation was carried out by peak area ratio measurements using a Spectra Physics, SP 4100 integrator/plotter. The coefficient of variation of caffeine determination in plasma was 13%.

Indocyanine green (ICG). ICG was measured by photometric assay [8] at a wavelength of 800 nm.

Serum bile acids. Total conjugated primary bile acids in serum were determined by radioimmunoassay (Becton Dickinson, Orangeburg, NY, USA).

Calculations

Disappearance rate constants (k) for caffeine and ICG were determined by log linear regression analysis of their respective concentration vs. time. From the intercept at time zero, the distribution volume (V_d) was calculated. Clearance (Cl) was defined as the product of k and V_d. Results are expressed as means ± SD. The statistical significance of differences between means was tested by the Mann-Whitney rank test. $P < 0.05$ was regarded as statistically significant.

Results

Serum bile acids and ICG clearance. The patients with cirrhosis and also those with non-cirrhotic liver disease differed significantly from the group of healthy volunteers with regard to fasting serum bile acids ($P < 0.01$) and ICG clearance ($P < 0.01$) (Table 2).

Fasting plasma caffeine. With the exception of one healthy volunteer and one patient with non-cirrhotic liver disease, caffeine could be detected (concentrations above 0.3 μmol/l) in the fasting plasma of all 27 subjects studied. The mean fasting plasma caffeine concentration was significantly ($P < 0.001$) higher in patients with cirrhosis (11.1 ± 10.5 μmol/l) than in healthy volunteers (1.5 ± 0.8 μmol/l). Of nine patients with cirrhosis, eight had an abnormal fasting plasma caffeine concentration which was defined as a value higher than two standard deviations above the mean of healthy controls. The fasting plasma caffeine concentrations in the patients with non-cirrhotic liver disease (3.1 ± 3.1 μmol/l) did not differ significantly from the healthy volunteers nor from the cirrhotics. After a 4-day caffeine abstinence, no caffeine could be detected in the healthy volunteers and in the patients with non-cirrhotic liver disease but in three patients with cirrhosis levels of 4–7 μmol/l were measured.

Caffeine elimination test. Mean caffeine concentrations of 14.2 ± 3.2, 9.2 ± 3.4 and 6.5 ± 1.7 μmol/l were attained in plasma 3 h after oral administration of 140 mg of caffeine in cirrhotics, patients with non-cirrhotic liver disease and healthy controls, respectively. Thereafter, caffeine disappeared from plasma according to a single exponential function (Fig. 1). The distribution volume of caffeine calculated per kg body weight tended to be smaller in cirrhotics, but this difference was not statistically significant (Table 2). Both plasma disappearance rate and clearance of caffeine were significantly decreased in cirrhotics ($P < 0.001$) and in patients with non-cirrhotic liver disease ($P < 0.025$). Of all parameters of caffeine elimination

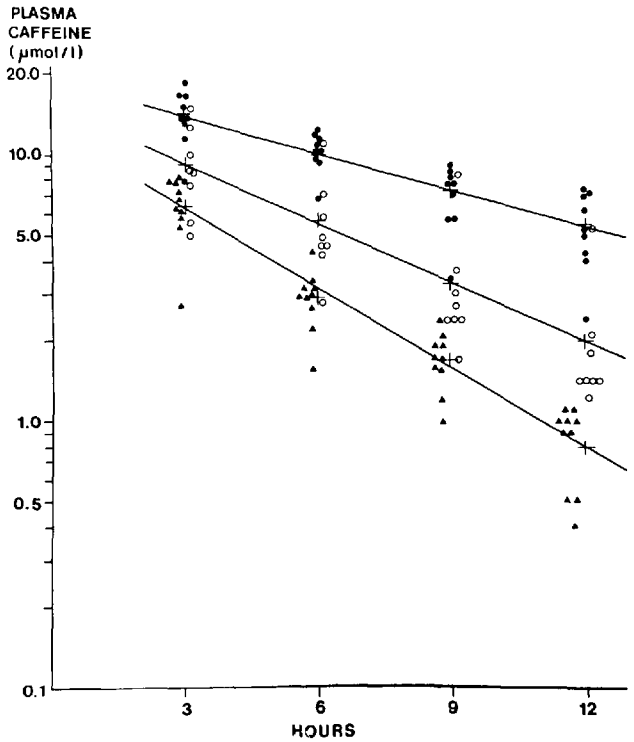

Fig. 1. Caffeine disappearance from plasma in patients with cirrhosis of the liver (●), patients with non-cirrhotic liver disease (○) and healthy volunteers (△). The means of the different groups are represented by crosses (+)

studied, plasma caffeine concentration determined 12 h after administration of the test dosage discriminated best beween the patients with liver disease and healthy volunteers. It was abnormal in all cirrhotics and in all patients with non-cirrhotic liver disease (Table 2).

Discussion

Since caffeine is ingested nearly ubiquitously with many beverages and foodstuffs, it is not surprising to find measurable plasma concentrations of caffeine in most subjects of our population. It has been shown that patients with liver disease tend to have higher fasting plasma levels of caffeine than healthy subjects and it had been suggested to use this parameter for evaluation of hepatic function [9]. However, an overlap of the fasting plasma caffeine concentrations has been observed between patients with liver cirrhosis and healthy volunteers [9]. This must be expected, if caffeine intake varies between the different individuals of the population studied. This may have been the case in a recent study by Mooney et al. [7] in Australia who could not confirm the results reported by Renner et al. [9] from Berne where the individuals that were studied may have shared a more common and regular pattern of caffeine intake.

The results of this study show that caffeine clearance or a single caffeine plasma concentration determined 12 h after oral administration of a test dose of caffeine in a patient who had been on caffeine abstinence are more reliable parameters of microsomal liver function and discriminated better between patients with liver disease and healthy controls than the fasting plasma caffeine concentration. This may primarily be explained by interindividual differences in caffeine consumption in our population. If caffeine intake prior to the test is standardized, measurement of one single caffeine concentration is sufficient.

Our findings in two groups of patients with liver disease which differed with regard to structural integrity and functional impairment of the liver confirm previous reports [4, 10] that caffeine is a useful compound for measuring liver function. Caffeine is a safe test compound that can be administered orally, is nearly exclusively metabolized by the liver [2] and can accurately be measured in serum. Liver function tests based on caffeine elimination may be regarded as complementary to ICG elimination tests and serum bile acid levels since caffeine elimination depends mainly on cytochrome P-448 mediated hepatic microsomal demethylation [2] whereas ICG elimination and serum bile acid levels depend on hepatic uptake and biliary excretion [8]. The simplicity of a single caffeine determination in plasma 12 h after a standardized dose of caffeine makes this test attractive for evaluation of liver function in clinical routine. It may be expected, that the introduction of a newly developed enzyme immunoassay [12] will further facilitate its performance.

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