Caffeine Clearance by Two Point Analysis: a measure of liver function in chronic liver disease

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This study attempted to compare the pharmacokinetic parameters of caffeine in patients with chronic liver disease and in normal subjects and to define the two sampling times which are suitable for determining caffeine clearance in these patients. Ten decompensated and eight compensated cirrhotic patients, and nine patients with chronic hepatitis were given a 3.5 mg/kg single oral dose of caffeine, followed by measurement of serum caffeine concentrations at 0, 30, 60, 90 minutes and 3, 5, 10, 24 and 36 hours using the HPLC technique. Caffeine clearance and its elimination rate constant in the decompensated cirrhotic patients were significantly lower than those in the compensated cirrhotic patients and much lower than in normal subjects (p<0.01). Caffeine clearance in chronic hepatitis patients was also significantly lower than in normal subjects. The volumes of distribution of caffeine in compensated and decompensated cirrhotic patients and normal subjects were significantly different. There was also a significant difference between normal subjects and the chronic hepatitis group. Serum caffeine clearance showed a good correlation with Child Pugh's score at r = -0.788. Two sampling times within 10 to 24 hours after oral dose of caffeine served as the best sampling points for determination of caffeine clearance by the simple equation; Cl = Kel *Vd (Vd is a fixed value in each group). It was clearly shown that caffeine clearance, calculated by two point analysis, would be a simple and useful method for measuring liver function in chronic liver disease.

(Key words: Caffeine clearance, Liver function, Chronic liver disease)

INTRODUCTION

Conventional liver function tests, such as AST, ALT, albumin and prothrombin time, do not represent the actual function of the liver. In recent years there have been increasing efforts to develop new tests which precisely reflect and quantify the liver’s metabolic function. The metabolic capacity of the liver can be quantified by measuring clearance rates of several compounds which are almost completely metabolized by the liver. Many tests were developed for routine use, but none has yet been found appropriate. The tests such as the aminopyrine breath test, galactose elimination, and bromosulphthalein disappearance, are not widely applied in daily clinical practice because of technical difficulties or adverse effects of the test reagents.

Caffeine (1,3,7-trimethylxanthine) is a non-toxic substance. It is rapidly and completely absorbed when taken orally. It is almost exclusively metabolized in the liver by demethylation in the cytochrome P450 mixed function oxidase system, which is the most important functional enzyme system of the liver. Its liver clearance is clearly classified as that of a capacity-limited and binding insensitive drug such as aminopyrine. It is an inexpensive compound and can be easily analyzed after administration either in plasma or saliva samples. Therefore, caffeine seems to be an almost ideal substance for routine assessment of liver metabolic function.

Caffeine clearance appears to show ethnic variation according to cytochrome P450
dependent metabolism, as reported in Thai (19) and normal Caucasian subjects (3, 6). It has been reported that the elimination of caffeine is delayed in patients with hepatic dysfunction (4). In this study, we compared the caffeine clearance values between normal subjects and patients with chronic liver diseases in order to determine whether the clearance can be used as a novel parameter for a liver function test. For routine laboratory analysis, we determined two sampling time points which are good for determining caffeine clearance.

MATERIALS AND METHODS

Subjects
Ten male and eight female patients with cirrhosis and seven male and two female patients with biopsy proven chronic hepatitis were hospitalized and participated in the study. Among the cirrhotic patients, nine were diagnosed as alcoholic cirrhosis, seven as post-hepatitis cirrhosis and two as cirrhosis of unknown origin. These patients were divided into two groups (10 decompensated and eight compensated cirrhosis) on the basis of their clinical and biochemical data and Child Pugh’s scoring system (12) (Table 1). Eight subjects with chronic hepatitis were diagnosed as chronic active hepatitis (CAH) infected with viral hepatitis B or C, and one as chronic persistent hepatitis (CPH). They all gave their informed consent to take part in the study.

The patients were asked to abstain from caffeine-containing beverages, foods and medication from 3 days before and throughout the study period.

Reagents
Caffeine (anhydrous, BP grade, batch no. 71015), 0.35% aqueous solution, was used for oral administration. 8-Chlorotheophylline, used as the internal standard, was purchased from Sigma Chemical Co. Ltd.; Zinc sulfate from Mallinckrodt Chemical Works; methanol and acetonitrile HPLC grade from Fison and FSA Laboratory Supplies; and sodium acetate from Fluka Chemie. Double-distilled water was used throughout this investigation.

Apparatus
HPLC apparatus consisted of a model 510 pump (Waters Associates, Milford, MA, USA) for delivering the mobile phase, a model Rheodyne injector for injection of samples, a Novapak C18 stainless steel column (particle size 5 μm, 15 cm 3.9 mm. I. D. Waters Associates) preceeded by a guard column filled with Corasil C-18 37-50 μm particles. A UV spectrophotometer (Model 481, Waters Associates) was used to monitor caffeine at a wavelength of 273 nm. An integrating recorder (Model 740, Waters Associates) was used to record the absorbance.

Methods
After overnight fasting, each subject took a 3.5 mg/kg single dose of caffeine orally. Blood samples were subsequently collected at 0, 30, 60, 90 minutes and 3, 5, 10, 24 and 36 hours following administration. The sera were separated and stored at −20°C until assayed.

Analytical procedure
Five hundred microliters of each serum sample was deproteinized using 100 μl of zinc sulfate solution (10% W/V), and 750 μl of methanol containing 4 μg/ml of the internal standard, 8-chlorotheophylline. Each sample
was vortex-mixed for 30 seconds and then centrifuged for 5 minutes at 4,000 rpm. The supernatant was filtered and then 50 µl of this filtrate solution was injected into the HPLC system\(^\text{[19]}\).

**Pharmacokinetic and Statistical Analysis**

Pharmacokinetic parameters of caffeine: \(C_{\text{max}}, T_{\text{max}}, K_e, V_d\) and \(C_l\); were calculated by a computer-based MKMODEL kinetic program\(^\text{[7]}\). Caffeine can be considered to be completely absorbed. The plasma concentration-time profile of caffeine can be applied to the one-compartment open model and exponential elimination declined. Caffeine clearance \((C_l)\) was calculated by two point analysis using the equation \(C_l = K_e - V_d\). \(K_e\) was determined from the slope of two points and \(V_d\) was obtained from the mean value in each group.

Significant differences in kinetic data were analyzed by Student's test or ANOVA and Duncan's New Multiple Range test at a level of significance of 0.05 or 0.01. The approximate value of caffeine clearance calculated by two point analysis was compared with the actual value from profile kinetic curve by Student's test. The correlation between caffeine clearance and Child Pugh's score was statistically determined at a level of significance of 0.05 or 0.01.

**RESULTS**

**Subjects**

Clinical and laboratory results of all subjects are summarized in Table 1. The average ages in the compensated and decompensated groups were not statistically different. In the compensated cirrhotic group, seven patients had no ascites and only one had mild ascites. The severity scored by Child Pugh's system was 5 to 7. In the decompensated group, all patients had slight to massive ascites. Their biochemical data were much higher than the normal values. The severity by Child Pugh's scoring system was also high at 8 to 14. The average age of the chronic hepatitis group (39.3) was not much higher than that of the normal group (32.7). Their laboratory data was close to normal except for AST and ALT. Ascites was absent in all cases.

After caffeine administration, no subject showed any clinical signs of toxic effects.

**Pharmacokinetic Data**

The elimination phase of caffeine among the normal, compensated and decompensated groups was significantly different as shown in Fig. 1. Each group also had obviously different kinetic data as demonstrated in Table 2. The absorption rate of caffeine was determined by the value of time to peak level \((T_{\text{max}})\) and peak level \((C_{\text{max}})\). It was rapid in compensated cirrhosis with \(T_{\text{max}}\) occurring between 0.5 and 1.5 hr. There was no significant difference in \(T_{\text{max}}\) or \(C_{\text{max}}\) between the compensated cirrhotic patients and the normal subjects. However, caffeine was absorbed more slowly in the decompensated cirrhotic patients than in the normal subjects. Caffeine clearance and its elimination rate constant in the decompensated group were significantly lower than in the compensated group and much lower than in the normal subjects \((p<0.01)\) (Table 2, Fig. 2). This indicated that there was dramatic impairment of caffeine clearance in patients with decompensated cirrhosis. Volumes of distribution \((V_d)\) of caffeine in the decompensated cirrhotic, compensated cirrhotic and the normal subjects were significantly different \((p<0.05)\). The degree of hepatic dysfunction assessed by Child Pugh's scoring system and the values of serum caffeine clearance were significantly correlated with a correlation coefficient \((r)\) of ~0.788 at \(p<0.01\) as shown in Fig. 3.

Caffeine clearance in the chronic hepatitis group was significantly lower than that in normal subjects \((p<0.05)\) but not as low as in the cirrhotic groups (Fig. 2). The elimination half life was also prolonged in this group (Table 2). It was evident that there was some impairment of liver function in these patients. \(V_d\) in chronic hepatitis patients was statistically different from that in normal subjects, which indicated a physiological change in these patients. The absorption phase as shown by \(T_{\text{max}}\) and \(C_{\text{max}}\) was not statistically different from that in normal subjects. It was as rapid as in the normal group. (Table 2)

The blood sampling times of 10 and 24 hr were chosen for determination of \(K_e\). The mean value of \(V_d\) in each group was used for the calculation. Clearance value was calculated from the equation \(C_l = K_e - V_d\). The clearance determined by two point analysis was not significantly different from the actual value as
Fig. 1 Kinetic profile of serum caffeine concentration-time curve in normal group and the patients with compensated, decompensated cirrhotic and chronic hepatitis groups after 0.35 mg/kg oral dose.

Table 2 Pharmacokinetic parameters of caffeine in normal subjects and patients*

<table>
<thead>
<tr>
<th>Subject</th>
<th>Cmax (µg/ml)</th>
<th>Tmax (hr)</th>
<th>Kel (hr⁻¹)</th>
<th>T1/2 (hr)</th>
<th>Vd (L/kg)</th>
<th>Cl (ml.min⁻¹kg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (n=20)</td>
<td>6.70±1.43</td>
<td>0.86±0.48</td>
<td>0.11±0.03</td>
<td>7.0±2.5</td>
<td>0.56±0.07</td>
<td>1.02±0.31</td>
</tr>
<tr>
<td>Compensated cirrhosis (n=8)</td>
<td>6.05±0.72</td>
<td>0.69±0.35</td>
<td>0.04±0.02+</td>
<td>20.2±11.0+</td>
<td>0.69±0.11+</td>
<td>0.46±0.17+</td>
</tr>
<tr>
<td>Decompensated cirrhosis (n=10)</td>
<td>4.71±0.68+</td>
<td>2.05±1.74+</td>
<td>0.01±0.01+</td>
<td>122.0±104.9+</td>
<td>0.84±0.14+</td>
<td>0.15±0.13+</td>
</tr>
<tr>
<td>Chronic hepatitis (n=9)</td>
<td>7.26±1.40</td>
<td>0.72±0.42</td>
<td>0.07±0.01+</td>
<td>10.58±2.52+</td>
<td>0.69±0.27++</td>
<td>0.78±0.20++</td>
</tr>
</tbody>
</table>

* Values are mean ± SD
+ significant difference from normal group p<0.01
++ significant difference from normal group p<0.05

shown in Table 3.

**DISCUSSION**

In this study, we attempted to investigate the pharmacokinetics of caffeine in patients with chronic liver disease. The results demonstrated that the kinetic profile of caffeine in cirrhotic and chronic hepatitis patients was different from that in normal subjects.

In the absorption phase; as in the normal subjects, caffeine was rapidly absorbed from GI tract in the compensated cirrhotic patients, with a peak in the blood about 40 min after administration, the same as in the chronic hepatitis group. However, in decompensated cirrhotic patients, the absorption rate of caffeine was slower than that in the other groups. This factor may prolong the serum level of caffeine in this group.

Caffeine is extensively metabolized in the liver. It is initially demethylated to dimethylxanthines by the hepatic microsomal
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**Fig. 2** Comparison of caffeine clearance between normal subjects and each patients groups: compensated, decompensated cirrhosis, and chronic hepatitis.

**Fig. 3** The relationship between caffeine clearance and Child Pugh's score in cirrhotic patients.

$r = -0.788 \ p < 0.01$
cytochrome P450 dependent mixed function oxidase system.\textsuperscript{2, 13} It is classified as a capacity-limited or a low clearance compound\textsuperscript{17}. Its clearance is dependent upon hepatic microsomal enzyme activity and is independent of liver blood flow\textsuperscript{11}. It is known that parenchymal liver diseases can cause impairment of elimination of a number of drugs metabolized by the mixed function oxidase system including caffeine\textsuperscript{1, 5, 11}. This leads to the suggestion that caffeine might be an ideal test substance for assessing hepatic function.

Similar to many drugs metabolized by cytochrome P450, caffeine metabolism is interethnically variable in oriental and caucasian groups\textsuperscript{6}. The pharmacokinetic parameters of caffeine metabolism in normal Thai subjects were previously studied\textsuperscript{19}. These parameters were used in this study to compare the caffeine metabolism in patients with chronic liver disease. All subjects in the study did not receive any drugs that can inhibit caffeine metabolism, including cimetidine, oral contraceptives and norflloxacin\textsuperscript{2}.

The results demonstrated that caffeine clearance in cirrhotic patients was significantly lower than in normal subjects. These data have been confirmed by other studies\textsuperscript{4, 9, 14}. Although the clearance in the decompensated cirrhotic group was tenfold lower than normal group, it was one half of that in the compensated group. This obviously suggests that liver function in decompensated cirrhotic patients was significantly impaired. It has been reported that concentrations and activities of hepatic drug metabolizing compounds are significantly reduced in patients with severe and extensive hepatocellular necrosis\textsuperscript{15}. The impairment of caffeine clearance observed in this study most likely resulted from reduction of the “functioning hepatocyte mass”\textsuperscript{14}. These decompensated cirrhotic subjects had clinically severe liver disease and most of them had abnormal laboratory data. However, the clinical and laboratory data did not define any significant changes in liver function in compensated cirrhosis. On the other hand, caffeine clearance might imply some significant impairment of liver function in these patients. It was also found that caffeine clearance revealed some liver impairment in the chronic hepatitis group with laboratory data were basically within normal limits. Therefore, the caffeine clearance may be a more sensitive index of the hepatic functional state than the conventional tests.

There was a significant linear correlation between caffeine clearance and the degree of hepatic dysfunction assessed by Child Pugh’s scoring system in the cirrhotic patients. However, the correlation in each patient showed a wide deviation from the group as a whole. The reason might be that many parameters are used for scoring the severity of liver disease by Child Pugh’s scoring system. Some parameters susceptible to variation due to individual clinical judgements\textsuperscript{12}.

We also investigated the two appropriate sampling times for determining caffeine clearance with the equation \( Cl = Kel - Vd \). The result demonstrated that biological sampling at 10 and 24 hr or two other times within 10 to 24 hr, after caffeine ingestion was appropriate.
to represent the elimination phase of caffeine. In the previous study, the plasma half life of caffeine in normal Thai subjects was 7 hr\(^{19}\). It was prolonged in the patients in this study. Our sampling times were within the actual elimination period of caffeine. In the equation above, a fixed Vd value for each group was used because of the significant difference of Vd in each group. This may be due to the physiological change seen in patients. In cirrhotic patients, the plasma protein binding of caffeine was lower than in normal subjects\(^4\). Ascites in decompensated cirrhosis directly affects the Vd value. These results were reported only in decompensated cirrhotic patients\(^15\). More subjects must be recruited to confirm the actual value of Vd for populations of compensated and decompensated cirrhotic and chronic hepatitis patients.

The caffeine level in saliva samples also showed good correlation with the serum caffeine level\(^{16,19}\). Therefore, saliva samples at two sampling times may be better than serum samples because of convenience and the non-invasive technique.

CONCLUSION

The highly significant difference of caffeine clearance between normal subjects and patients with chronic liver disease obviously shows that it is highly sensitive for determination of liver dysfunction. Caffeine clearance is much more sensitive than conventional liver function tests. It should be useful as a marker to assess liver function.

Caffeine clearance determined by two point analysis at 10 and 24 hr or two other times within 10 to 24 hrs after caffeine ingestion, the best sampling times, provides a simple and useful method for measuring liver function in chronic liver disease.

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