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# Caffeine clearance as a measure of liver function in cirrhotic patients

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*This study attempted to compare the pharmacokinetic parameters of caffeine in decompensated and compensated cirrhotic patients with normal subjects and to define the two sampling times which are most suitable for determining caffeine clearance in cirrhotic patients. Ten decompensated and seven compensated cirrhotic patients were given a 3.5 mg/kg single oral dose of caffeine, and this was followed by measuring their serum caffeine concentrations at 0,30,60,90 minutes and 3, 5, 10, 24 and 36 hours using the high-performance liquid chromatographic (HPLC) technique. Caffeine clearance and elimination rate in the decompensated cirrhotic patients were significantly lower than in the compensated cirrhotic ones and much lower than in normal subjects ($p<0.01$). The volume of distribution of caffeine in the decompensated, compensated cirrhotic patients and normal subjects were significantly different from each other ($p<0.05$). Serum caffeine clearance has a good correlation with the Child Pugh score at $r = - 0.810$. The two sampling times at 10 and 24 hours after the oral dose of caffeine served as the best sampling points for determining caffeine clearance by the simple equation; $Cl = Kel \times Vd$ (Vd is a fixed value in each group). It is clearly shown that caffeine clearance calculated from two point (10 and 24 hrs) analysis would be a simple and useful method for measuring liver function in cirrhotic patients.*

**Key words :** *Caffeine clearance, Cirrhotic patients, Liver function.*

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**สุพีชา วิทยเลิศปัญญา, สัจพันธ์ อิศรเสนา, โสภิต ธรรมอารี, เพ็ญศรี ทองนพเนื้อ, ปิยะวัฒน์ โกมลมิตร, วันดี ชานุตโร. การตรวจสอบการทำงานของตับในผู้ป่วยโรคตับแข็ง โดยใช้คาเฟอีนเคลียร้านซ์ จุฬาลงกรณ์เวชสาร 2538 มกราคม;39(1): 19-27**

*ศึกษาเปรียบเทียบค่าทางเภสัชจลนศาสตร์ของคาเฟอีนในผู้ป่วยโรคตับแข็งชนิดรุนแรงและไม่รุนแรงเทียบกับคนปกติ และหาจุดตัวอย่างเลือด 2 จุด ที่เหมาะสมต่อการหาค่าคาเฟอีนเคลียร้านซ์เพื่อใช้ตรวจสอบการทำงานของตับในผู้ป่วย โดยให้ผู้ป่วยโรคตับแข็งชนิดรุนแรง 7 ราย และชนิดไม่รุนแรง 10 ราย รับประทานยาคาเฟอีนขนาด 3.5 มก./กก. เจาะเลือดที่เวลา 0, 30, 60, 90 นาที 3, 5, 10, 24 และ 36 ชั่วโมงหลังรับประทานยา วิเคราะห์หาระดับยาคาเฟอีนในซีรั่มในแต่ละเวลาโดยใช้วิธีเอชพีแอลซี ผลการทดลองพบว่าค่าคาเฟอีนเคลียร้านซ์และค่าคงที่ในการกำจัดยาในผู้ป่วยโรคตับแข็งชนิดรุนแรง มีค่าน้อยกว่ากลุ่มผู้ป่วยโรตตับแข็งชนิดไม่รุนแรง และมีค่าน้อยกว่าคนปกติอย่างมีนัยสำคัญทางสถิติที่ระดับ 0.01 ค่าปริมาตรการกระจายตัวของคาเฟอีนในกลุ่มผู้ป่วยโรคตับแข็งชนิดรุนแรง ชนิดไม่รุนแรง และกลุ่มคนปกติ มีความแตกต่างกันอย่างมีนัยสำคัญทางสถิติที่ระดับ 0.05 ความสัมพันธ์ระหว่างค่าคาเฟอีนเคลียร้านซ์กับค่า Child Pugh score ซึ่งบ่งบอกระดับความรุนแรงของโรคมีความสัมพันธ์กันที่ระดับ r = -0.810 การเก็บตัวอย่างเลือดที่เวลา 2 จุด คือ 10 และ 24 ชั่วโมง สามารถใช้คำนวณหาค่าคาเฟอีนเคลียร้านซ์จากสูตร CL=KelxVd โดยใช้ค่าเฉลี่ยปริมาตรการกระจายตัวของแต่ละกลุ่มในการคำนวณ ซึ่งผลที่ได้ไม่แตกต่างจากค่าจริง การใช้วิธีนี้ในการประมาณหาค่าคาเฟอีนเคลียร้านซ์จึงเป็นวิธีที่เหมาะสมต่องานบริการการตรวจสอบการทำงานของตับในผู้ป่วยโรคตับแข็ง*

Conventional liver function tests such as SGOT, SGPT, albumin and prothrombin time do not represent actual function of the liver. In recent years there have been increasing efforts to develop new tests which more 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 have been developed for routine use, but none has yet been found to be appropriate. The tests such as the aminopyrine breath test[1], galactose elimination[2], and bromosulphophthalein disappearance are not routinely accepted in daily clinical practice because of technical difficulties or adverse effects of the tested drugs.

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 a system of demethylation with cytochrome P450 mixed function oxidase system and this is the most important functional enzyme system of the liver.[3] Its liver clearance is clearly classified as a capacity-limited and binding insensitive drug as aminopyrine.[4] It is an inexpensive compound and simply assayed in plasma or saliva.[5,6] Therefore, caffein seems to be an almost ideal substance for the routine assessment of liver metabolic function.

Caffeine clearance seems to have variation among the races according to cytochrome P450 dependent metabolism, as reported in Thai[5] and caucasian normal subjects.[7] It has been reported that the elimination of caffeine is delayed in patients with hepatic dysfunction.[8] In this study, we compared the caffeine clearance values between normal subjects and patients with decompensated and compensated cirrhosis in order to determine whether the clearance can be used as a novel parameter for liver function testing. It might also be a better parameter than Child Pugh's score which is used to evaluate the degree of severity in cirrhotic patients. For routine laboratory analysis, we find that two sampling time points which serve as good timing points for determining caffeine clearance.

## Materials and Methods

### Subjects

Ten male and seven female patients hospitalized with biopsy-proven cirrhosis, age ranged 27-68 years, participated in the study. Nine subjects were diagnosed as having alcoholic cirrhosis, six as post-hepatitis cirrhosis and two as cirrhosis of unknown origin. All patients were divided into two groups (10 decompensated and 7 compensated cirrhosis) on the basis of their clinical and biochemical data and the Child Pugh's scoring system[9] (Table 1). They all gave their informed consent to take part in the study.

**Table 1.** Clinical and laboratory data in cirrhotic patients.*

| Subject | Sex (N) | Age | Weight | AST | ALT | Total bilirubin | Albumin | PT | Ascites | Child Pugh's score |
|---|---|---|---|---|---|---|---|---|---|---|
| | (yrs) | (kg) | (U/L) | (U/L) | (mg/dl) | (g/dl) | (sec) | | | (5-15) |
| | Normal values : | | 0-38 | 0-38 | | 0.3-1.2 | 3.4-5.5 | 11-14 | | |
| Compensated cirrhosis | F(2),M(5) | 51.0±14.3 (27-66) | 57.5±6.5 (47-69) | 53±25 (29-93) | 39±14 (16-54) | 0.9±0.4 (0.4-1.8) | 3.4±0.5 (2.8-4.4) | 12.0±1.3 (10.1-14.3) | Absent to slight | 5-6 |
| Decompensated cirrhosis | F(5),M(5) | 48.1±9.9 (30-59) | 56.3±12.6 (31-77) | 104±74 (43-283) | 34±14 (10-61) | 10.2±10.6 (9.78-33) | 2.3±0.4 (1.5-2.9) | 19.4±4.3 (14.2-28) | Absent to Massive | 8-14 |

* Values are means ± SD followed by (ranges)

The patients were asked to abstain from caffeine-containing beverages, foods and medication 7 days before and throughout the study period.

### Reagents

Caffeine (anhydrous, BP grade, batch no.71015), 0.35% aqueous solution, was use 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, FSA Laboratory Supplies; and sodium acetate from Fluka Chemical. Double-distilled water was used throughout this investigation.

### Apparatus

The HPLC apparatus was composed of a model 510 pump (Waters Associates, Milford, MA, USA) for delivering the mobile phase; a model Rheodyne injector for injecting samples, and a Novapak C18 stainless steel column (particle size 5μm, 15 cm x 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 wavelength 273 nm. An integrating recorder (Model 740, Waters Associates) was used to record the absorbance.

## Methods

After an overnight fasted, 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 the administration. The sera were seperated and were stored at -20° C until assayed.

### Analytical Procedure

A 500 μl 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 sytem.[5]

### Pharmacokinetic and Statistical Analysis

The pharmacokinetic parameters of caffeine: Cmax, Tmax, Kel, Vd and Cl; were calculated by a computer-based MKMODEL kinetic program.[10] Caffeine can be considered to be completely absorbed. The plasma concentration-time profile of caffeine can be fitted with one-compartment open medel and exponential elimination declined. Caffeine clearance (Cl) was calculated from two point analysis by using the equation of Cl= Kel x Vd. Kel was determined from the slope of two points and Vd was obtained from the mean value in each group. Significant differences in kinetic data were analyzed by ANOVA and Duncan's New Multiple Range test. The approximate value of caffeine clearance calculated from two point analysis was compared with the actual value from the profile kinetic curve by the Student's T test. The correlation between caffeine clearance and Child Pugh's score was statistically determined at significant level of 0.05.

## Results

### Subjects

The clinical and laboratory results of all subjects are summarized in Table 1. The average ages of the compensated and the decompensated groups were not statistically different. In the compensated cirrhotic group, six patients were absent of ascites and only one had a mild degree of ascites. The degrees of severity scored by the Child Pugh's scoring system were 5 to 6. In the decompensated group, all patients had slight to massive ascites. Their biochemical data were much higher than normal value. The degree of severity as determained by the Child Pugh's scoring system was also high at 8 to 14.

No subjects showed any clinical sign of toxic effects after caffeine administration.

### Pharmacokinetic Data

The caffeine elimination phase among the

normal, the compensated and the decompensated groups were 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 levels (Tmax) and peak level (Cmax). It was rapid in the compensated cirrhotic group with Tmax occurring between 0.5-1.5 hr. There was no significant difference in Tmax or Cmax 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. The caffeine clearance and its elimination rate constant in the decompensated group was significantly lower than in the compensated group and much lower than in normal subjects ($p<0.01$). This indicated that there was dramatic impairment of caffeine clearance in the patients with decompensated cirrhosis. The volume of distribution (Vd) of caffeine in the decompensated cirrhotic subjects, the compensated cirrhotic subjects and the normal subjects were significantly different between groups ($p<0.05$). The degree of hepatic dysfunction assessed by the Child Pugh's scoring system and the values of serum caffeine clearance was significantly correlated with the correlation coefficient value (r) of - 0.810 at $p<0.01$, as shown in Fig 2.

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

| Subject | Cmax (μG/ml) | Tmax (hr) | $T_{1/2}^{+}$ (hr) | $Vd^{++}$ (L/kg) | $Cl^{+}$ ($ml.min^{-1}kg^{-1}$) |
|---|---|---|---|---|---|
| Normal (n=20) | 6.70±1.43 | 0.86±0.48 | 7.0±2.5 | 0.56±0.07 | 1.02±0.31 |
| Compensated cirrhosis (n=7) | 5.92±0.73 | 0.64±0.38 | 19.7±11.8 | 0.68±0.08 | 0.48±0.17 |
| Decompensated cirrhosis (n=10) | $4.72±0.72^{+++}$ | $2.05±1.83^{+++}$ | 114.5±104.5 | 0.84±0.15 | 0.15±0.12 |

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

The blood sampling times at 10 and 24 hours were chosen for determining of Kel. The mean value of Vd in each group was used for calculation. Clearance value was calculated from the equation of Cl = Kel x Vd. The clearance determined from two point analysis was not significantly different from the actual values, as shown in Table 3.

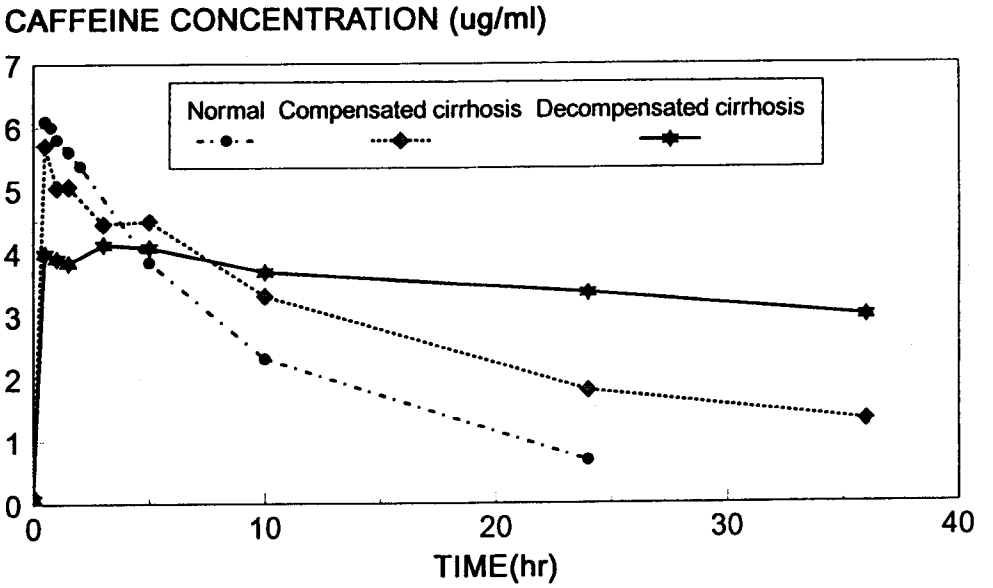


**Figure 1.** Kinetic profile of serum caffeine concentration-time curve in normal subjects and patients with compensated and decompensated cirrhosis after 0.35 mg/kg oral dose.

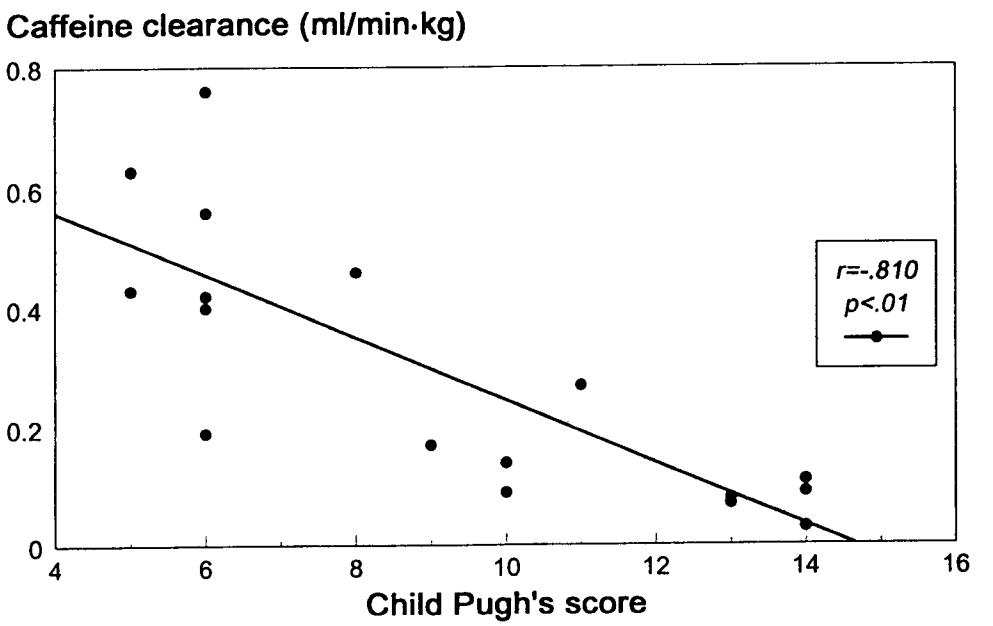


**Figure 2.** The relationship between caffeine clearance and Child Pugh's score in cirrhotic patients.

**Table 3.** Comparison of the value of caffeine clearance calculated from profile curve and from two points at 10 and 24 hr in each group.

| Subjects | Cl (ml/min.kg) from profile curve | Cl (ml/min.kg) from two points |
|---|---|---|
| Normal (N=18) | 0.98 ± 0.30 | 0.98 ± 0.29 |
| Compensated cirrhosis (N=7) | 0.48 ± 0.18 | 0.58 ± 0.38 |
| Decompensated cirrhosis (N=10) | 0.16 ± 0.13 | 0.16 ± 0.19 |

*Values are mean ± SD

**The data are not statistical different in each group. ($p > 0.05$)

## Discussion

In this study, we wanted to investigate the pharmacokinetics of caffeine in cirrhotic patients. The results demonstrated that the kinetic profiles of caffeine in these patients were different from normal subjects.

In the absorption phase, caffeine was rapidly absorbed from the GI tract in the compensated cirrhotic patients, with a peak in the blood about 40 min after administration. This was similar to normal subjects. In decompensated cirrhotic patients, the absorption rate of caffeine was slower. 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 cytochrome P450 dependent mixed function oxidase system.[3,11] It is classified as a capacity-limited or a low clearance compound.[4] Its clearance is dependent upon hepatic microsomal enzyme activity and is independent from liver blood flow.[12] It has been known that parenchymal liver diseases can cause impairment in the elimination of a number of drugs metabolized by the mixed function oxidase including caffeine[12]. 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 intereth-nically variable between oriental and caucasian groups.[7] The pharmacokinetic parameters of caffeine metabolism in normal Thai subjects were previously studied.[5] Those parameters were used in this study for comparison with caffeine metabolism in cirrhotic patients. All subjects in our study did not receive any drugs that could inhibit caffeine metabolism, including cimetidine, oral contraceptives or norfloxacin.[11]

The results demonstrated that caffeine clearance in cirrhotic patients was significantly lower than in normal subjects. This data corresponds with that of other studies.[8,13,14] While the clearance in the decompensated cirrhotic group was one-tenth that of the normal group, the clearance in the compensated group was half that of the normal group. This obviously suggests that the liver function in decompensated cirrhotic patients was significantly impaired. It has been reported that the concentrations and activities of hepatic drug metabolizing compounds are significantly reduced in patients with severe and extensive hepatocellular necrosis.[16] The impairment in caffeine clearance observed in this study most likely resulted from reduction in "functioning hepatocyte mass". These decompensated cirrhotic subjects had clinically severe liver disease and most of them had abnormal

laboratory data. However, the clinical and laboratory data could not define any significant changes in liver function in compensated cirrhosis. On the other hand, the caffeine clearance could imply some significant impairment of liver function in these patients. Therefore, caffeine clearance may be a more sensitive index of hepatic functional state than the conventional liver tests.

There was a significant linear correlation between caffeine clearance and the degree of hepatic dysfunction as assessed by the Pugh's score rating system in cirrhotic patients as a group. However, the correlation in each patients was widely scattered throughout the group. This might be due to the large number of parameters used for scoring the severity of liver disease by the Child Pugh's scoring system. Some parameters are easily varied due to individual clinical judgement.[9]

We also investigated two appropriate sampling time points for determining caffeine clearance with the equation of Cl = Kel x Vd. The result demonstrated that samples at 10 and 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.[5] It was prolonged in cirrhotic patients in this study. Our sampling time points are in the actual elimination period of caffeine. In the equation above, the fixed Vd value for each group was used because of the significant difference of Vd in each group. In cirrhotic patients, the plasma protein binding of caffeine was lower than normal subjects.[8] Ascites developed in decompensated cirrhosis subjects directly affects the Vd value. These results were reported only in decompensated cirrhotic patients.[16] More studies are needed to confirm the actual value of Vd for populations of compensated and decompensated cirrhotic patients.

Furthermore, the caffeine level in saliva samples showed good correlation with serum caffeine level.[5] There fore, for more convenience, saliva samples may be better than serum samples.

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