Caffeine Clearance Study in Hepatocellular Carcinoma

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Abstract

The purpose of this study was to evaluate hepatic metabolic capacity in cirrhotic patients with hepatocellular carcinoma (HCC). We compared plasma caffeine clearance, calculated by two point analysis, between patients with cirrhosis alone and cirrhosis complicated with HCC. These two groups were comparable with regards to age, sex, and the severity of liver disease, graded by Child-Pugh score as compensated and decompensated cases. From our result, caffeine clearance in compensated cases was clearly higher than that of decompensated cases in both groups studied, particularly in the HCC group ($p=0.001$). The mean value of caffeine clearance in HCC patients correlated well with the tumor staging as classified by Okuda's criteria. There was also a reversal correlation between tumor size and the clearance tested in compensated cases of HCC ($p=0.046$), but this finding was not detected in decompensated cases ($p>0.05$). We conclude that the determination of caffeine clearance can serve as a useful parameter for the asssessment of hepatic functional reserve in cirrhotic patients complicated with HCC, and may be a useful predictor for survival outcome.

Key word : Caffeine Clearance, Cirrhosis, Hepatocellular Carcinoma

Hepatocellular carcinoma (HCC) constitutes one of the most common cancers in the world. The etiology of HCC appears to be multifactorial, however, more than 90 per cent of HCC develops in pre-existing liver cirrhosis(1). As a result, the prognosis of HCC is determined not only by the tumor stage, but also by the functional status of the underlying chronic liver disease(2). Therefore, evaluation of the hepatic functional reserve may be useful in the therapeutic management of these patients.

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Biochemical measurements, such as AST, ALT, albumin and prothrombin time, provide a crude assessment of the degree of liver injury, but do not represent the actual residual liver reserve. In contrast, the administration of a compound metabolized by the liver provides a dynamic evaluation of residual metabolic capacity[3]. Many tests have been developed for routine use, such as the aminopyrine breath test, galactose elimination, and bromosulphothalein disappearance, but none has yet been found appropriate in clinical practice because of technical difficulties or adverse effects of the test compounds.

Caffeine clearance is a reliable indicator of global hepatic function[4,5] and it is useful in assessment of the severity of cirrhosis[6], that has the advantage of simplicity of performance and analysis. It can simply be analyzed in plasma, or in saliva samples[5,7-9]. Furthermore, caffeine is a non-toxic substance, well absorbed when taken orally and almost completely metabolized in the liver by cytochrome P450 isoenzyme-mediated N-demethylation[10]. Therefore, it seems to be an almost ideal test for routine assessment of liver metabolic function.

In order to determine the effect of HCC on hepatic metabolic capacity, we compared the caffeine clearance values between patients with cirrhosis alone and cirrhosis complicated with HCC. In this study, we assessed the caffeine elimination rate in plasma samples by using a simplified method, calculated by two-point analysis as mentioned elsewhere[11].

MATERIAL AND METHOD

Population Study

Twenty three patients with cirrhosis and twenty two patients with histologically- proven HCC and cirrhosis, having attended Chulalongkorn University Hospital between January and April 1998, were included in the study. They were informed as to the objective of the study and subsequently provided their consent. The patients were asked to abstain from smoking, caffeine-containing beverages, foods and medications that can inhibit caffeine metabolism, for example cimetidine, oral contraceptives and norfloxacin[12] at least 3 days before and throughout the study period.

Table 1 summarizes the main characteristics of the subjects. There was no difference between these two groups with regard to age, sex, and underlying etiologies as well as the severity of liver disease, graded as compensated cases (Child-Pugh score 5-7) and decompensated cases (Child-Pugh score 8-15). Among the HCC patients, there were 12 cases with the tumor's largest diameter smaller than 10 cm and the remaining cases had a tumor size larger than 10 cm. The Okuda's stage of tumor using functional parameters (ascites, albumin, bilirubin) and tumor size were graded as stage I in three, stage II in twelve and stage III in seven patients, respectively.

Reagents

Caffeine (anhydrous, BP grade, batch no. 71015), 0.35 per cent aqueous solution, was used for oral administration. Zinc sulfate was purchased from Mallinckrodt Chemical Works; methanol and acetonitrile HPLC grade from Fison and FSA Laboratory Supplies; and sodium acetate from Fluka Chemic. Double-distilled water was used throughout this investigation.

Apparatus

HPLC apparatus consisted of a mode l P1000 SpectraSystem (Thermoseparation pro-

Table 1. Characteristics of patients included in the study.

	Age (yr)	M/F	Etiology		Child's score		Tumor size(cm)		OKUDA staging		
			viral*	non-viral	5-7	8-15	<10	>10	I	II	III
Cirrhosis (n=23)	55.7±10.5 (30-73)	16/7	14	9	14	9	--	--	--	--	--
HCC (n=22)	55.3±9.3 (35-75)	19/3	15	7	13	9	12	10	3	12	7
	NS	NS	NS		NS						

NS = not statistically significant.
viral = positive for HBV and/or HCV serum markers

ducts) for delivering the mobile phase, a model automatic injector AS3000 for injection of samples, a Novapak C 18 stainless steel column (particle size 5 , 15 cm -3.9 mm. I. D. Waters Associates) UV detector with a model of UV 1000 was used to monitor caffeine at a wavelength of 273 nm. A computer system with PC1000 software was used to analyse peak and set the standard system.

Method

After an overnight fast, each subject took a 3.5 mg/kg single dose of caffeine orally. Blood samples were subsequently collected at 12 and 18 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),mixed 10 second and followed by 750 μl of absolute methanol. 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[11].

Pharmacokinetic and Statistical Analysis

Caffeine clearance (CI) was calculated by two point analysis using the equation CI=Kel x Vd. Kel was determined from the slope of two points and Vd was obtained from the mean value in each patient group classified by a previous report[11].

Differences in caffeine clearance in the study groups were analyzed by Mann-Whiney U test and Kraskal Wallis test at a significant level of 0.05.

RESULTS

Caffeine clearance from the two study groups is shown in Fig. 1. In the cirrhosis group, the mean value of caffeine clearance in compensated patients was 0.50 ± 0.17 ml/min/kg, which was higher than that found in decompensated cases (0.22 ± 0.08 ml/min/kg). In the HCC group, there was a significant difference between compensated (0.60 ± 0.21 ml/min/kg) and decompensated cases (0.09 ± 0.05 ml/min/kg P=0.001). However, among cirrhosis and HCC patients with the same range of Child's score, the clearance was not significantly different (P>0.05).

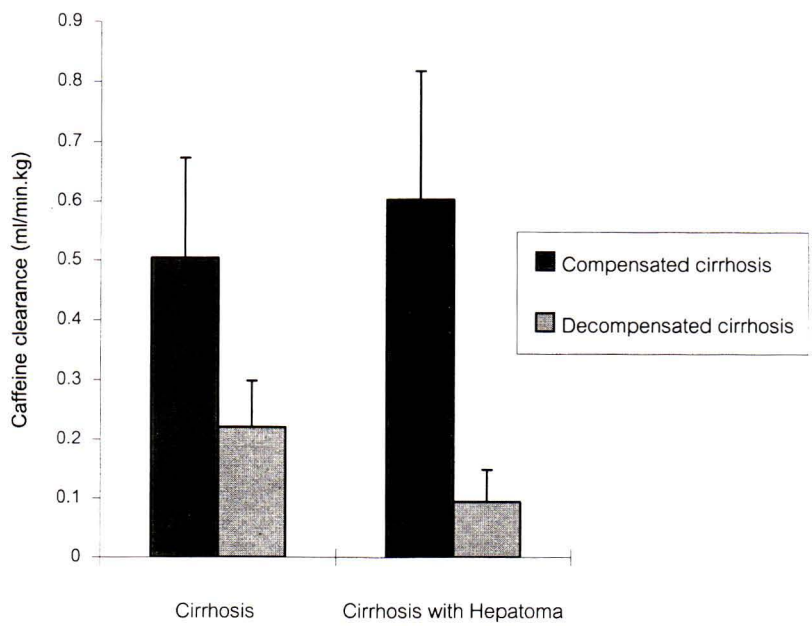

Fig. 1. Caffeine clearance from the two studied groups.

In HCC patients, there was a good correlation between caffeine clearance and tumor staging classified by Okuda's criteria (Fig. 2). The clearance in Okuda stage I was 0.91 ± 0.27 ml/min/kg, which was higher than those of stage II (0.37 ± 0.18 ml/min/kg) and stage III (0.11 ± 0.08 ml/min/kg). In contrast, the correlation between caffeine clearance and tumor size alone was not consistent. The clearance in compensated cases with smaller tumor size (0.96 ± 0.16 ml/min/kg) was significantly greater

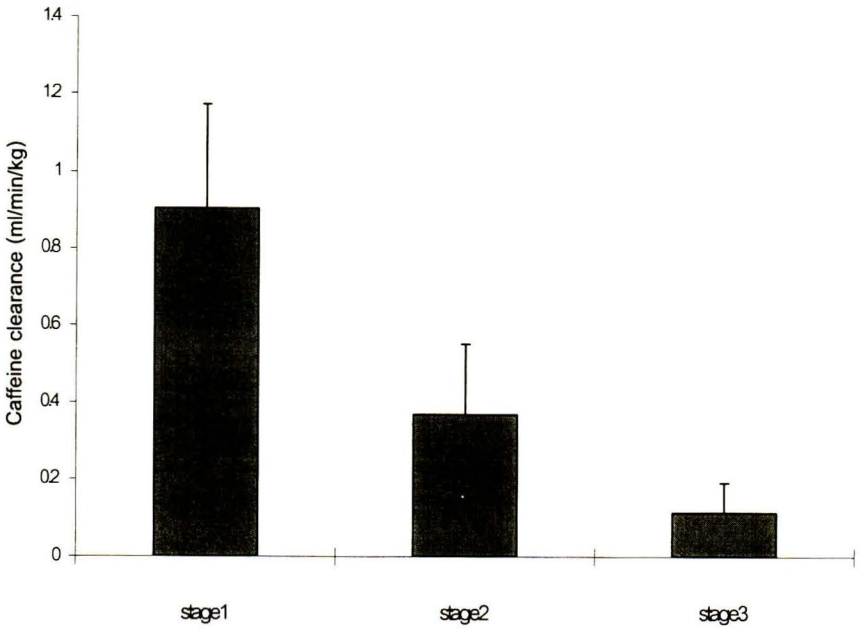

Fig. 2. Caffeine clearance in difference staging of HCCas classified by Okuda's criteria.

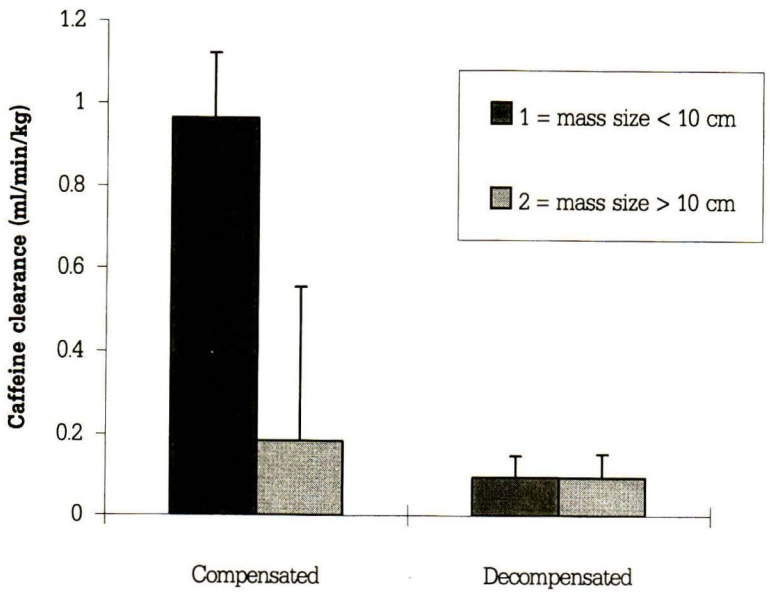

Fig. 3. Caffeine clearance in HCC with difference tumor sizes.

than cases with a larger tumor burden (0.18 ± 0.37 ml/min/kg) (P=0.046), whereas, in decompensated cases, the clearance was comparable regardless of tumor size (0.010 ± 0.05 ml/min/kg for tumor size < 10 cm. and 0.09 ± 0.06 ml/min/kg for tumor size > 10 cm, P>0.05). (Fig. 3).

DISCUSSION

Caffeine is a capacity-limited or low clearance compound which is independent of alteration in hepatic blood flow, whereas, changes in liver cells functionally able to metabolize the substance have a large effect on clearance[14]. Its elimination depends highly on cytochrome P450IA2 isoenzyme-mediated N-demethylation, which leads to a variety of urinary methyxanthine metabolites[10]. The elimination half-life of caffeine ($t_{1/2}$) varies from 3-7 hours in healthy adults and is not affected by increasing age or body weight[15,16]. However, drug interaction may influence results, for example, cimetidine decreases clearance but barbiturates and many other drugs, including smoking increase the activity and can act as a confounding variable[17]. The subjects in our group were nonsmokers and did not receive the above-mentioned drugs during study.

Several methods have been used to quantitate liver function with caffeine. The conventional [^{13}C] caffeine breath test is highly specific for CYPIA2 isoenzyme activity, which catalyzes caffeine 3-N-demethylation[18]. Alternatively, caffeine or caffeine metabolites can be analyzed in fasting plasma, urine, or saliva by HPLC that eliminate the requirement for radioactivity and breath collections. But these methods also monitor metabolites that are produced by several cytochrome P450 isoforms, including CYPIA2, CYPIIIA4, and CYPIIA6 (18). From our previous study using the HPLC method[11], we found that the best two sampling times to represent the elimination phase of caffeine were between 10 to 24 hours after ingestion. By this method, it is more practical to perform the overnight caffeine clearance test in hospitalized, as well as ambulatory patients.

The result of the present study demonstrated a correlation between caffeine clearance and the degree of liver impairment assessed by Child Pugh score in both groups of patients. The clearance rate was also comparable between the two groups who had the same range of Child Pugh score. Considering all patients, the clearance in decompensated cirrhosis was about threefold lower than that of compensated cases. The impairment of the test most likely resulted from reduction of the functioning hepatic metabolic capacity[17]. Although it is unclear whether caffeine clearance measurement is superior to the traditional Child-Pugh score in predicting prognosis[19], several studies have documented that it is more sensitive for detecting the severity of liver disease[5,7,10].

Among the HCC patients, caffeine clearance also correlated with the tumor staging according to Okuda's criteria, the most widely used prognostic classification. This staging scheme had the merit of emphasising the importance for prognostic purposes of the association between underlying cirrhosis and neoplastic disease. As a result, the mean survival time of patients in stage I was better than those of stage II and III[20]. From our data, the elimination rate of caffeine in patients with stage I was higher than those of stage II and III, about three-fold and nine-fold higher, respectively. Therefore, the caffeine clearance test seems to be a good functional marker for assessment of the prognosis of liver cirrhosis complicated with HCC.

In HCC cases with a similar range of Child-Pugh score, there was no consistent reversal correlation between tumor size and the clearance tested. As we expected, in compensated cases, it appeared that the smaller the tumor size, the higher the caffeine elimination rate. This result could be easily explained by the fact that a larger tumor mass would reduce more functioning hepatic metabolic reserve and the test was sensitive enough for determination of liver dysfunction. However, this observation did not exist in decompensated cases because it was shown that various sizes of tumor masses, large or small, made no difference to the clearance rate of caffeine.

The pathophysiological mechanisms through which caffeine clearance were not different among different tumor sizes in decompensated cases were entirely speculative, but could include the following : (i) the sample size included in the study was too small to discriminate the difference. (ii) the sensitivity of the test was not satisfactory enough to determine liver impairment in far-advanced cirrhosis with HCC.(iii) there could be substantial variations of the parameters in the Child Pugh scoring system from individual judgement[21]. (iv) the liver functional capacity did not depend on tumor size

alone in far-advanced cases (v) there might be a reflection of altered microsomal metabolism within the tumor tissues as indicated in mouse models that liver tumors invariably overexpressed CYPIIA forms[22,23]. Although recent data did not confirm this pattern in human HCC samples, there might be tumor subgroups that were distinguished by CYPIIA expression[24].

In conclusion, this study revealed that hepatic microsomal enzyme activity, as shown by the caffeine clearance test, was reversibly impaired in decompensated cirrhosis as well as in the advanced stage of HCC. The impaired elimination of caffeine was due to changes in hepatic metabolism paralleled alterations in "functional liver cell metabolic capacity". Besides its safety and simplicity of performance determined by two point analysis, it should be taken into consideration to be a complementary functional staging in management of patients with cirrhosis complicated with HCC and a predictor to measure the survival and outcome of patients with HCC.

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การศึกษาการทำงานของตับในผู้ป่วยโรคมะเร็งตับ โดยใช้ค่าคาเฟอีนเคลียร้านซ์

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ได้ทำการศึกษาเพื่อประเมินการทำงานของตับโดยใช้วิธี caffeine clearance ในผู้ป่วยโรคตับแข็งที่มีและไม่มีโรคมะเร็งตับชนิด hepatocellular carcinoma (HCC) โดยทั้งสองกลุ่มที่ศึกษาไม่มีความแตกต่างกันในเรื่อง อายุ, เพศ, สาเหตุของการเกิดตับแข็งและระดับความรุนแรงของโรคโดยใช้เกณฑ์ตาม Child-Pugh score แบ่งผู้ป่วยเป็นกลุ่มที่การทำงานของตับยังอยู่ในเกณฑ์ดี (compensated cirrhosis) และกลุ่มที่การทำงานของตับไม่ดี (decompensated cirrhosis) ผลการศึกษาพบว่า ค่าของ caffeine clearance ในกลุ่มที่การทำงานของตับยังอยู่ในเกณฑ์ดีมีค่าสูงกว่าเมื่อเทียบกับกลุ่มที่การทำงานของตับไม่ดีโดยเฉพาะในผู้ป่วยที่มีโรคมะเร็งตับร่วมด้วย ($p = 0.001$) ค่าของ caffeine clearance ในผู้ป่วยที่มีมะเร็งตับมีความสัมพันธ์กับระยะความรุนแรงของโรคตามเกณฑ์ของ Okuda's criteria นอกจากนี้ในกลุ่มที่เป็นมะเร็งตับที่การทำงานของตับยังอยู่ในเกณฑ์ดี พบว่าผู้ป่วยที่มีขนาดของก้อนมะเร็งเล็กกว่า 10 เซนติเมตรจะมีค่าของ caffeine clearance สูงกว่ากลุ่มผู้ป่วยที่มีขนาดของก้อนมะเร็งใหญ่กว่า 10 เซนติเมตร อย่างมีนัยสำคัญ ($P = 0.046$) แต่อย่างไรก็ตามไม่พบความสัมพันธ์นี้ในกลุ่มที่การทำงานของตับไม่ดี ($P > 0.05$) จากการศึกษานี้สรุปได้ว่าการวัด caffeine clearance มีประโยชน์ในการประเมินการทำงานของตับในผู้ป่วยโรคตับแข็งที่มีโรคมะเร็งตับร่วมด้วยและอาจมีประโยชน์ในการนำมาใช้เพื่อการพยากรณ์โรคต่อไป

คำสำคัญ : คาเฟอีนเคลียร้านซ์, ตับแข็ง, มะเร็งตับ

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