

Full Length Research Paper

Preoperative evaluation of hepatic reserve function by phenacetin metabolism test for the prevention of postoperative liver dysfunction

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Accepted 16 July, 2012

The aim of this study was to investigate the utility of the phenacetin metabolism test in evaluating hepatic reserve function prior to hepatectomy for the prevention of postoperative liver dysfunction. Fifty-six patients with hepatocellular carcinoma had undergone hepatectomy. Patients were classified into group I (normal group, n = 37) and group II (with peak total bilirubin >53.8 $\mu\text{mol/L}$ for 7 days after hepatectomy, n = 19) based on the levels of total bilirubin after hepatectomy. The receiver operating characteristic (ROC) analysis was made to assess the hepatic reserve function to predict liver dysfunction of the patients after hepatectomy. Hepatic reserve function was evaluated by phenacetin metabolism test; the ratio of plasma total paracetamol to phenacetin at 2 h after oral 1.0 g phenacetin. There were no significant differences in preoperative variables or intraoperative findings except the ratio of plasma total paracetamol to phenacetin. ROC analysis showed that the sensitivity and specificity of the ratio of plasma total paracetamol to phenacetin ≤ 1.2 were 85.4 and 72.9%, respectively for predicting liver dysfunction of the patients after hepatectomy. The ratio of plasma total paracetamol to phenacetin correlated with the temporary postoperative liver dysfunction (P = 0.008). Phenacetin metabolism test before hepatectomy appears to provide direct and reliable measure of hepatic reserve function, thus helping in surgical decision making regarding the extent of hepatectomy and in the prevention of the occurrence of postoperative liver dysfunction.

Key words: Phenacetin metabolism test, hepatic reserve function, hepatectomy, liver dysfunction.

INTRODUCTION

Although, less than 30% of the patients used in this study are eligible for liver resection, either due to tumor multifocality or severity of their underlying liver disease, surgery remains the most effective treatment in patients with hepatocellular carcinoma (HCC) (Schwartz et al., 2007; Llovet et al., 2005). The function of the underlying liver clearly plays an important role in the early postoperative outcome. Hepatic reserve function is the

most crucial factor to consider in planning the extent of hepatic resection (Mullin et al., 2005). Precise evaluation is particularly important before resection for hepatoma, because almost all the patients are infected with hepatitis B or C virus and have chronic liver disease.

Child-Pugh score has been the traditional gold standards for assessment of hepatic reserve function since 1973 (Pugh et al., 1973), but appears to be sensitive to

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changes in severity of liver disease (Garello et al., 1999). Although, several methods have been used to quantitatively evaluate the hepatic reserve function, including clearance of indocyanine and breath tests, a definitive method is yet to be established. Liver volume and shape may reflect hepatic reserve function (Shoup et al., 2003). But it is very difficult to accurately estimate the remnant liver volume using the ratio of liver section, because the range of variation is very large under different conditions (Abdalla et al., 2004). The linear index of oral glucose tolerance test (OGTT) is related to the mitochondrial activity of the hepatocytes and is useful for the diagnosis of early-stage cirrhosis, but the factor of age may influence the result (Sasaya et al., 2000). Measures of hepatic indocyanine green (ICG) kinetics are widely used to evaluate hepatic reserve function, especially in patients who have already undergone hepatic resection (Nonami et al., 1999; Ren et al., 2012). The test results are unreliable, however, in cases of jaundice, when a portosystemic shunt is present, or when blood collection times cannot be observed strictly. The ^{13}C -methacetin breath test can serve as a non-invasive alternative for evaluating liver reserve prior to hepatectomy, but hypoxia and endogenous CO_2 production changes will affect its accuracy (Ilan, 2007).

Phenacetin O-deethylation is a marker reaction for CYP1A2 activity (Fukami et al., 2007). With a very low extrahepatic metabolic rate of less than 5% (Cui et al., 2002), phenacetin has been used as a probe drug to assess liver function (Qu et al., 2007; Huang et al., 2008). The clinical value of phenacetin metabolism test for evaluating human liver reserve function was confirmed in patients with liver cirrhosis (Xiong et al., 2010). However, there is little report about the application of phenacetin metabolism test in the preoperative evaluation of hepatic reserve function in patients with hepatocellular carcinoma (HCC). The purpose of this study is to evaluate the clinical value of phenacetin metabolism test in the assessment of hepatic reserve function before hepatectomy for HCC.

MATERIALS AND METHODS

From October, 2009 to June, 2011, a total of 56 patients who had undergone liver resection for HCC at our department were enrolled in this study. Written informed consent was obtained from all patients. The protocol of the study was approved by the Medical Ethics Committee of the First Affiliated Hospital of Zhengzhou University, and the study was performed in accordance with the ethical standards established in the 1964 Declaration of Helsinki.

There were 37 men and 19 women, with a mean age of 46.2 ± 10.6 years (range 26 to 69 years) in the study. The diagnosis of HCC was made in majority of patients, based on histology and cytology of pathological specimens. In rare cases, diagnosis of HCC was based on the presence of a liver tumor with a serum α -fetoprotein value exceeding 250 ng/ml. Among the patients, there were 35 Child-Pugh in class A and 21 in class B. The surgical modalities included trisectionectomy for 15 patients, bisegmentectomy for 29, and partialresection for 12.

All patients underwent blood tests and conventional simple liver

function tests before resection and during days 1, 3, and 7 after resection. Patients were classified into two groups according to the levels of total bilirubin (TB) after hepatectomy. Postoperative temporary liver dysfunction was defined as a peak, $\text{TB} > 53.8 \mu\text{mol/L}$ for 7 days after hepatectomy (Akita et al., 2008). The clinical course of the patients was followed up for a minimum of 1 month after hepatectomy. Eight patients got a phenacetin test more than 1 month later after hepatectomy.

Phenacetin metabolism test

One week before the study, phenacetin analgesics, smoking, cruciferous vegetables, roasted meats, and drugs that affect the activity of P450-1A₂, such as omeprazole, carbamazepine, theophylline, verapamil, cimetidine, and quinolones, were prohibited. The subjects went on an overnight fast and orally took 1.0 g phenacetin with 200 ml warm water the next morning. No food and drinks were allowed for 2 h after intake. The blood sample (2 ml) was taken from the antecubital vein at 2 h after oral administration of phenacetin. All samples were stored at -20°C until further measurement. The test was repeated in these HCC patients during days 1, 3, and 7 after hepatectomy.

Analysis of phenacetin and paracetamol

Blood samples were digested with β -glucuronidase/arylsulfatases (Sigma, St. Louis, USA) at 40°C overnight. They were concentrated in glass columns (1.5×10 cm) packed with washed XAD-161 resin. Then, they were washed with 10 ml of deionized water and eluted with 10 ml of methanol. The eluates were injected into the high performance liquid chromatography (HPLC) system for analysis. A Waters X-Terra C18 column was used with a mixture of acetonitrile (0.01 mol/L): aminic acid (30:70, v/v) as the mobile phase and a detector set at 254 nm. Phenacetin and its metabolites (free-, glucuronide- and sulfate-paracetamol) were determined by HPLC. The ratio of plasma paracetamol to phenacetin was calculated.

Statistical analysis

Data were presented as means \pm standard error of mean (SEM) and Statistical Package for Social Sciences (SPSS) 13.0 software was used for statistical analysis. Statistical comparisons were made using non-parametric Mann-Whitney U or Dunnett's t-test. Correlation and partial correlation analysis were conducted by linear regression analysis. Chi-square test was used to compare discrete variables. For the determination of the receiver operating characteristic (ROC) curve, ROC curve analysis software was used (MedCalc, version 7.5.0.0). Statistical significance was defined as $P < 0.05$.

RESULTS

Preoperative ratio of plasma paracetamol to phenacetin in 35 Child-Pugh A class patients and in 21 Child-Pugh B class patients were 1.14 ± 0.46 and $1.39 \pm 0.35\%$ respectively; there was a significant difference between the two groups ($P < 0.05$).

Thirty seven patients (66.07%) without peak $\text{TB} > 53.8 \mu\text{mol/L}$ for 7 days after hepatectomy had good clinical outcomes (group I), but 19 patients (33.93%) who experienced temporary postoperative liver dysfunction (with peak $\text{TB} > 53.8 \mu\text{mol/L}$ for 7 days after hepatectomy)

Table 1. Comparison of the variables in the two groups (mean \pm SD).

Variable	Group I (n = 37)	Group II (n = 19)	P value
Age (yr)	47.3 \pm 17.6	44.7 \pm 14.1	0.386
Child-Pugh score	5.24 \pm 0.58	5.61 \pm 0.93	0.079
MELD score	3.17 \pm 2.83	3.78 \pm 3.59	0.394
PT-INR	1.07 \pm 0.13	1.12 \pm 0.09	0.175
TB (μ mol/L)	14.92 \pm 7.16	17.86 \pm 12.43	0.284
ALB (g/L)	40.24 \pm 7.72	38.56 \pm 11.61	0.692
ALT (IU/L)	51.9 \pm 42.7	73.4 \pm 53.6	0.134
AST (IU/L)	57.6 \pm 49.2	78.4 \pm 57.1	0.163
ALP (IU/L)	104.4 \pm 63.8	131.7 \pm 52.0	0.141
GGT (IU/L)	165.3 \pm 154.6	225.9 \pm 138.1	0.158
Ratio of Pa/Ph	1.42 \pm 0.31	1.03 \pm 0.57	0.009
TLV (mL)	1325 \pm 374	1478 \pm 436	0.192

MELD score: the model for end-stage liver disease score; PT- INR: prothrombin time-international normalized ratio; TB: total bilirubin; ALB: albumin; ALT: alanine aminotransferase; AST: aspartate aminotransferase; ALP: alkaline phosphatase; GGT: gamma-glutamyl transferase; Ratio of Pa/Ph: the ratio of plasma total paracetamol to phenacetin; TLV: total liver volume.

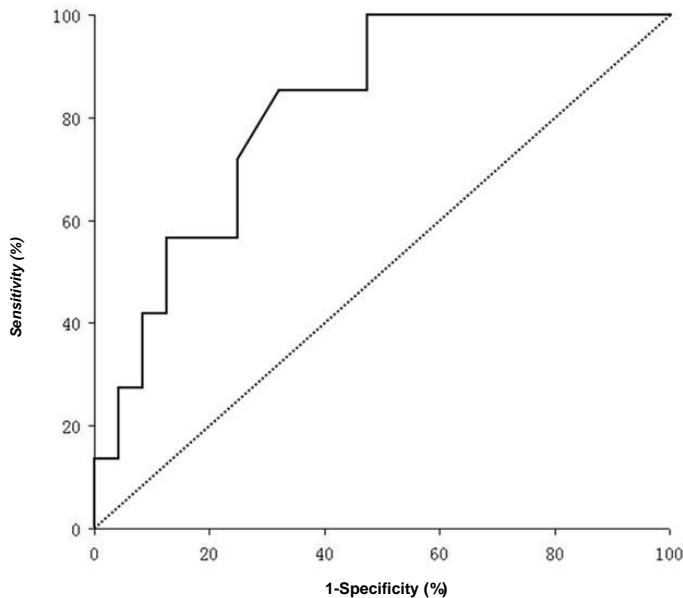


Figure 1. The sensitivity and specificity of the ratio of Pa/Ph \leq 1.2 were 85.4 and 72.9%, respectively, for predicting temporary postoperative liver dysfunction by ROC analysis.

needed prolonged intensive management, and showed poor clinical courses (group II). Of all the patients that recovered from temporary postoperative liver dysfunction, none of them died of liver failure. There were no differences in age, Child-Pugh score, model of end-stage liver disease (MELD) score, prothrombin time-international normalized ratio, TB, albumin (ALB), alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT),

and total liver volume (TLV) between the two groups ($P > 0.05$), but phenacetin test variables, the ratio of plasma total paracetamol to phenacetin, showed significant differences between group I and group II ($P = 0.009$; Table 1).

The critical ratio of plasma total paracetamol to phenacetin (Pa/Ph) value to predict temporary postoperative liver dysfunction was 1.2 according to ROC analysis (Figure 1). The sensitivity and specificity were 85.4 and 72.9%. Then, we divided patients into two groups according to this standard. A total of 20 patients had a ratio of Pa/Ph \leq 1.2, out of which 13 (65%) had temporary postoperative liver dysfunction defined as a peak TB $>$ 53.8 μ mol/L for 7 days after hepatectomy (Akita et al., 2008). In contrast, of the 36 patients with a ratio of Pa/Ph $>$ 1.2, only 6 (16.67%) had temporary postoperative liver dysfunction. The difference in the frequency of temporary postoperative liver dysfunction between these two groups was significant ($P = 0.008$; Table 2). The rate of temporary postoperative liver dysfunction was greater when ratio of Pa/Ph \leq 1.2.

DISCUSSION

Liver has a substantial regenerative ability and an immense reserve capacity. With improvement in operative and anesthetic techniques, and preoperative and postoperative management, the mortality of patients after liver resection has decreased. However, the incidence of postoperative liver failure is still high; liver failure is the major cause of morbidity and mortality after hepatectomy (Wu et al., 1998), and various methods have been proposed to estimate liver functional reserve before surgery (Gill et al., 1983; Schneider, 2004). In Japan, the

Table 2. chi square test comparing ratio of Pa/Ph and TB (P = 0.008).

TB	Ratio of Pa/Ph	
	≤1.2	>1.2
≤53.8 μmol/L	7	30
>53.8 μmol/L	13	6

ICG-R15 measured during hepatectomy is considered to evaluate the liver functional reserve, but this approach is controversial in other countries (Lam et al., 1999; Imamura et al., 2005). In fact, the ICG-R15 is used widely in the field of liver transplantation across Japan (Hori et al., 2006; Eguchi et al., 2009). However, hepatic reserve function was predicted in these studies from the combination of preoperative ICG concentration in the whole liver and liver volume measurement by computed tomography, whereas the actual function of residual liver could not be measured accurately.

It is well known that the first-pass effect and extrahepatic metabolism of phenacetin are extremely low. Mediated by CYP450 1A2, phenacetin becomes the phase I metabolite acetaminophen by oxidation of an ethyl group immediately upon entry into the human body. Monitoring CYP450 1A2-mediated phenacetin metabolism is a simple and efficient method for evaluating human hepatic reserve function (Xiong et al., 2010). Studies (Qu et al., 2007) of liver function suggested that phenacetin metabolism test (the ratio of total plasma acetaminophen to phenacetin) could actually reflect the damage of liver function.

The causes of liver failure are complex, because of broad differences in liver function, tumor size and location and operative procedures for each patient; thus, no single parameter provides sufficient data for a safe limit of hepatectomy. Preoperative assessment is critical when considering surgery for patients with HCC. Liver volume can reflect hepatic reserve, which can be used to assess the operative risk before hepatectomy and to guide the ideal treatment (Schiano et al., 2000; Li et al., 2003). In this study, we found that the ratio of Pa/Ph reduced after liver resection (P < 0.05), but there is no significant difference in the total liver volume between two groups (P > 0.05). This dissociation is probably associated with functional capacity of liver cells before resection, such as liver fibrosis due to viral hepatitis, etc. We think the ratio of Pa/Ph may estimate the liver function that included the hepatocytes, the hepatic metabolism, and the liver volume.

In present, liver reserve function is usually evaluated by means of Child-Pugh scores and MELD, including serum creatinine, bilirubin, international normalized ration of prothrombin time, and etiology of the underlying liver disease (Salerno et al., 2002). There were no differences in Child-Pugh score, MELD score between HCC patients with temporary postoperative liver dysfunction and HCC

patients without temporary postoperative liver dysfunction. It is probably associated with chronic hepatitis B and cirrhosis secondary to an HBV infection in most HCC patients in China. In the HCC patients with Child-Pugh class A, the damage to liver function is not reflected in the Child-Pugh classification (Huang et al., 2008).

Our results showed that higher ratio of Pa/Ph values were related with serious, though temporary, postoperative liver dysfunction. We calculated that the critical point of the ratio of Pa/Ph value was 1.2 according to ROC analysis. Then, the groups were divided according to the critical point and distinctive differences were found in temporary liver dysfunction posthepatectomy. There was higher rate of liver dysfunction when the ratio of Pa/Ph value was ≤1.2. Accordingly, careful postoperative management should be considered in patients with the ratio of Pa/Ph ≤1.2.

Similar to many other diagnostic tests, several issues remain unresolved in phenacetin metabolism test. CYP450 1A2 activity can be reduced with age, but not to a level that disrupts the normal metabolism of phenacetin (Xiong et al., 2010). Another major drawback of phenacetin metabolism test is invasively and time consuming for the patient, because the blood sample was taken from the antecubital vein at 2 h after oral administration of phenacetin.

conclusively, our findings indicate that phenacetin metabolism test can be used to predict postoperative hepatic reserve function in patients undergoing hepatic resection; although, further studies of larger samples are needed to validate these results. Careful patient selection based on phenacetin metabolism test in such major hepatectomy cases should help to prevent the occurrence of postoperative liver dysfunction.

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