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ORIGINAL ARTICLE

The effect of transcatheter arterial chemoembolization on phase II drug metabolism enzymes in patients with hepatocellular carcinoma

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Abstract
Purpose Transcatheter arterial chemoembolization (TACE) causes damage to liver function and decreases the activity of cytochrome P450 in patients with hepatocellular carcinoma (HCC). But there was no report on whether the activity of Phase II conjugating enzymes was affected in HCC patients after TACE treatment. The purpose of the study was to assess the effect of TACE on the activity of UDP-glucuronosyltransferases (UGTs) and sulfotransferases (SULTs) in HCC patients.
Methods The acetaminophen test was performed on 12 normal subjects and 26 HCC patients. The contents of acetaminophen and its metabolites in blood and urine were determined by HPLC method. The recoveries of acetaminophen glucuronide (AG) and acetaminophen sulfate (AS) in plasma and urine were investigated.
Results Compared with pre-TACE treatment, serum albumin decreased by 10.3%, bilirubin increased by 50.9%, prothrombin time was prolonged by 10.4%, serum alanine aminotransferase increased by 1.27-fold and aspartate aminotransferase increased by 1.29-fold in HCC patients after TACE ($p < 0.01$). But there was no significant difference on the contents of blood and urinary free and conjugated acetaminophen in HCC patients before and after TACE. Compared with normal controls, the ratio of urinary AG to AS was just 13.2% ($p < 0.01$) in HCC patients.
Conclusion TACE possesses apparent damage to liver function, but it does not affect the activity of UGTs and SULTs in HCC patients. The metabolism pathway of acetaminophen was altered for HCC patients: acetaminophen was metabolized mainly into AS for HCC patients but mainly into AG for healthy volunteers.

Keywords Transcatheter arterial chemoembolization (TACE) · UPD-glucuronosyltransferase · Hepatocellular carcinoma · Sulfotransferase

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Introduction

Hepatocellular carcinoma (HCC) is a major health problem worldwide, with an estimated new cases incidence ranging between 500,000 and 1,000,000 annually. It is the fifth most common neoplasm in the world, and the third most common cause of cancer-related death [1]. Although resection is the first choice of treatment for hepatic cancer, only 20–30% of patients have the opportunity to undergo resection. Transcatheter arterial chemoembolization (TACE) is a typical intervention therapy. Its advantages include minimal trauma, precise efficacy, and convenience of administration. Consequently, it has become a widely used treatment for patients who cannot undergo resection [2]. Such a treatment causes ischemic damage to the tumor due to the mechanical embolization of the artery feeding the neoplasm and prolongs the duration of the effects of chemotherapeutic agents [3]. It is well known that TACE caused damage to liver function and decreased the activity of cytochrome P450 (CYP450) in HCC patients [4–7].

However, there was no report on whether the activity of phase II conjugating enzymes was impacted in patients with HCC after the treatment of TACE.

Both the UDP-glucuronosyltransferases (UGTs) and sulfotransferases (SULTs) are superfamilies of phase II conjugating enzymes that play an important role in the elimination of a vast number of endogenous and exogenous substrates by the addition of a glucuronide or sulfate moiety [8, 9]. The glucuronide and sulfate conjugates are generally high-polar compounds and are ready excreted in the urine [10]. Acetaminophen (APAP) is a safe drug that has been used as an in vivo probe to determine the activity of UGTs and SULTs in human [11, 12]. When given in therapeutic doses, it is metabolized primarily by conjugation to form glucuronide and sulfate derivatives. The metabolites of APAP [APAP glucuronide (AG) and APAP sulfate (AS)] are makers for GST and UGT activity [13].

Patients and methods

Subjects

The study was conducted in 12 normal subjects and 26 HCC patients. Twelve normal subjects were healthy volunteers. Their health condition was measured based on their medical records and physical examination, vital signs, 24-h dynamic ECG monitoring, routine clinical laboratory tests (hematology, serum biochemistry, urinalysis, hepatitis B surface antigen screen, hepatitis C antibody screen, and HIV antibody screen). None consumed alcohol or tobacco. No drug was taken, including contraceptives, within 2 weeks during the test.

Twenty-six HCC patients were enrolled in the study. The characteristics of subjects were shown in Table 1. The diagnosis of HCC was based on the histological and cytological findings or on the presence of a liver tumor with a serum α-fetoprotein value exceeding 250 ng/mL. Patients were excluded if they had an indication for surgery, had been treated previously for HCC, had heart or kidney dysfunction, had contraindications for treatment with TACE, had a smoking habit or had a medication affecting the activity of phase II drug metabolism enzyme within 2 weeks during the test. Child's Class B and C patients, pregnant, or breastfeeding patients, those with serious uncontrolled concomitant systemic disorders or extrahepatic metastasis were also excluded.

The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Human Ethics Committee of Tangdu Hospital, Fourth Military Medical University, China. Written informed consent was obtained from each subject prior to the participation in the study.

Study design

An open-label clinical study was performed at Tangdu Hospital, Fourth Military Medical University. Volunteers were allocated into two groups: the normal control group enrolling healthy volunteers and the experimental group enrolling primary HCC patients who was scheduled to undergo TACE. All subjects were required to fast overnight. A single 1,000 mg dose of APAP was given to all subjects with 200 mL water in the morning. No food or drinks were allowed for 2 h following APAP administration. 10 mL of blood samples was drawn from antecubital vein and collected into tubes at 2 h after administration, and a 24-h urine specimen was collected in tubes after oral administration of APAP for 12 healthy subjects and 26 HCC patients. All samples were separated and kept −20°C until analysis. The test was repeated in HCC patients after TACE. After 2 days, the first APAP test, TACE was performed in HCC patients according to the protocol reported previously. The femoral artery was punctured and the catheter was inserted into the hepatic artery. The emulsified mixture of lipoidal, epirubicin, 5-FU, and carboplatin was injected into the tumor vessel. All TACE treatments on the HCC patients were conducted by the same team of radiologists.

There was an interval of 4 days after TACE treatment before the patients received the second APAP test. Clinical and laboratory data were recorded in detail. In this

Table 1 Characteristics of the subjects in this study ($\bar{x} \pm$ SD)

	Healthy subjects ($n = 12$)	HCC before TACE ($n = 26$)	HCC after TACE ($n = 26$)
Age (years)	45.4 ± 6.3	48.6 ± 11.7	As before
Gender (female/male)	4/8	5/21	As before
Body weight (kg)	64.1 ± 5.7	62.9 ± 10.3	60.6 ± 9.4
Child-Pugh class		A	A (23), B (3)
Tumor nodes		≥3 (9) and <3 (17)	As before
Maximum tumor node		≥3 cm (11) and <3 cm (15)	As before

study, the APAP test was based on the ratio of plasma and urine APAP metabolites to total APAP (unchanged APAP plus its glucuronide and sulfate conjugate forms) and the ratio is used as an index of hepatic phase II drug metabolism.

Drug assays

Acetaminophen and its metabolites (free-, glucuronide- and sulfate-APAP) in the biologic samples were measured using a modified HPLC method, aminophylline as an internal standard. AG and AS were assayed by selective enzymatic hydrolysis and followed by HPLC method [13]. AG and AS specimen were digested with β-glucuronidase/arylsulphatases (Sigma, St Louis, MO, USA) at 40°C overnight, and then was 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 HPLC system for analysis. A Waters X-Terra C_{18} column (Waters Corporation, Milford, MA, USA) was used with a mixture of acetonitrile and 0.01 mol/L methanoic acid (30:70, v/v) as the mobile phase, and a detector were set at 254 nm.

Statistical analysis

Data are presented as mean ± SD. Statistical comparisons between groups were made using non-parametric Mann–Whitney U test, chi-squared test or Dunnett's t test. The self-control data were compared using paired t test or Wilcoxon test. Multiple linear regression analysis was used to assess the influence of patients' clinical and laboratory variables on the ratio of urine AS and AG to total APAP. SAS (SAS Institute, Cary, NC, USA) software package was utilized in the analysis. Statistical significance was defined as $P < 0.05$.

Results

The contents of APAP and its metabolites in plasma and urine were showed in Figs. 1 and 2. The contents of plasma conjugated APAP were 6.3 ± 2.2, 6.0 ± 2.8, and 5.7 ± 1.6 μg/mL for HCC patients before and after TACE treatment and for healthy subjects, respectively. And there was no significant difference on the recovery of plasma free and conjugated APAP in HCC patients before and after TACE and in healthy volunteers.

The recoveries of urine total APAP were 64.3 ± 16.2, 67.4 ± 20.1, 70.7 ± 19.4% for HCC patients before and after TACE treatment and for healthy subjects, respectively. However, the ratio of urinary AG/AS was 0.19 ± 0.05, 0.18 ± 0.05 and 1.43 ± 0.46 for HCC patients before and after TACE and for healthy subjects, respectively. It was suggested that the metabolism pathway of APAP was altered and most of APAP was metabolized into AS for HCC patients but into AG for healthy volunteers. When compared with pre-TACE treatment, there was no significant difference in APAP metabolism in HCC patients, who had undergone treatment with TACE.

All subjects completed the study and none experienced any adverse effects, except liver function damage after TACE of anticancer drug in HCC patients. The data are shown in Table 2. All biochemical indices including, albumin, bilirubin, prothrombin time, ALT and AST, altered significantly ($P < 0.01$), especially ALT and AST. When compared with pre-TACE treatment, serum albumin decreased by 10.3%, bilirubin increased by 50.9%, and prothrombin time was prolonged by 10.4% for HCC patients who had undergone treatment with TACE. Although statistically different, the three indices were still in the normal range for the majority of patients. Serum alanine aminotransferase (ALT) increased by 1.27-fold and aspartate aminotransferase (AST) increased by 1.29-fold in HCC patients after TACE treatment ($P < 0.01$). Multivariate

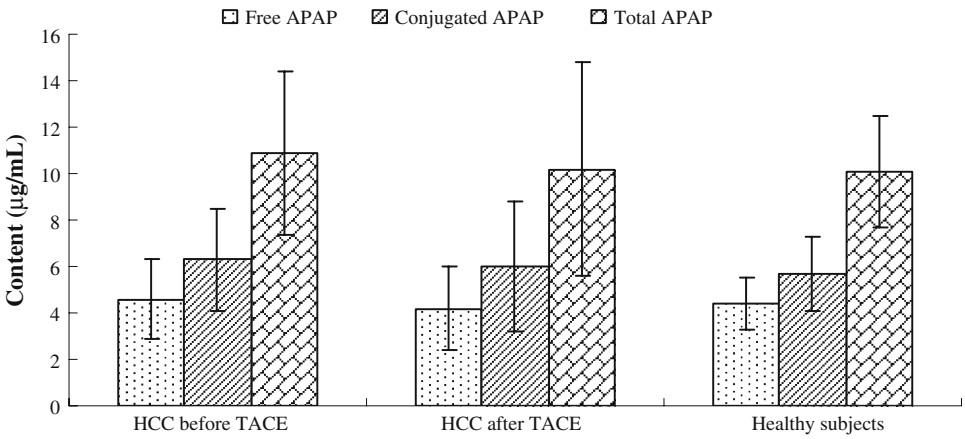

Fig. 1 Contents of APAP and its metabolites in plasma

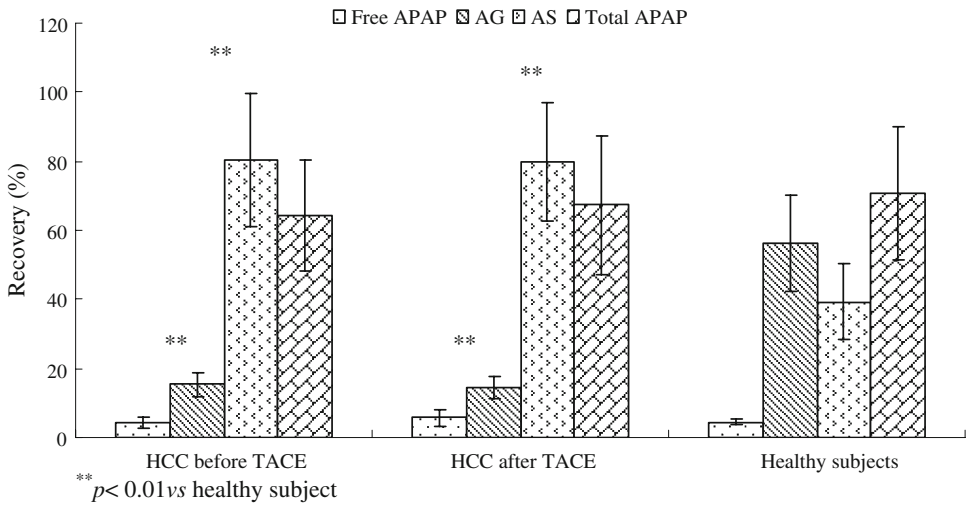

Fig. 2 The total urinary APAP excretion rates of APAP in urine and the ratio of free and conjugated APAP to total APAP in urine. The total urinary APAP excretion rates of APAP were 64.3 ± 16.2, 67.4 ± 20.1 and 70.7 ± 19.4% for HCC patients before and after TACE treatment, and for healthy subjects, respectively. And the ratios of free APAP to total APAP were 4.2 ± 1.5, 5.8 ± 2.5 and 4.5 ± 0.8%, the ratios of AG to total APAP were 15.3 ± 3.7, 14.4 ± 3.1 and 56.2 ± 14.1%, and the ratios of AS to total APAP were 80.5 ± 19.3, 79.8 ± 17.1 and 39.3 ± 10.9% in urine for HCC patients before and after TACE treatment, and for healthy subjects, respectively

Table 2 Changes of biochemical indices in HCC patients before and after TACE ($\bar{x}$ ± SD)

	Healthy subjects ($n = 12$)	HCC before TACE ($n = 26$)	HCC after TACE ($n = 26$)
Albumin (g/L)	49.4 ± 2.3	40.8 ± 6.1**	36.6 ± 5.2**·***
Bilirubin (μmol/L)	10.7 ± 3.5	13.1 ± 5.4	25.7 ± 14.2**·***
Prothrombin time (s)	12.1 ± 0.6	12.8 ± 1.5	14.3 ± 1.3*·***
ALT (U/L)	31.6 ± 6.3	57.4 ± 29.1**	130.5 ± 102.3**·***
AST (U/L)	22.8 ± 5.2	54.9 ± 37.3**	126.2 ± 92.4**·***

* $P < 0.05$ and ** $P < 0.01$ versus healthy subjects; *** $P < 0.01$ versus before TACE

analysis indicated that gender, age, body weight, and tumor size had no influence on the ratio of APAP metabolites (AG/AS) in urine.

Discussion

Acetaminophen, as a probe drug, is widely used to study the effect of genetic, environmental, and other factors on the activity of UGTs and SULTs in human and animals, because the drug is eliminated in the urine primarily as AS and AG with only 5% of a therapeutic dose being excreted unchanged [11]. Previous study indicated that acetaminophen (APAP) was safe as a probe drug in human at a single oral dose of 1,000 mg [12]. Some in vitro investigations indicate that the glucuronidation and sulfation, mediated by UGTs and SULTs, affect the actions and disposition of diverse endogenous and exogenous compounds in humans and animals, including steroids, fatty acids, catecholamine neurotransmitters, thyroid hormones, and propofol [13, 14].

The number of subjects in the study here is relatively small sample and the APAP test has been verified in HCC patients and HIV+/AIDS patients [12]. Therefore, further research is necessary on the clinical use of the APAP test in patients with various types of liver disease, relationship with hepatic histopathology need to be examined further and comparisons made with other methods for evaluating liver function.

In China, hepatitis B virus infection is most likely a high risk factor for HCC [15]. In HCC patients, with Child–Pugh class A, the impairment in liver function is not often reflected in the classification. Such patients may suffer from serious liver function impairment after TACE [6]. Therefore, we selected HCC patients with Child–Pugh class A to run the APAP test. The results showed that there was no significant difference in the plasma and urine

recovery of AG and AS in HCC patients between pre- and post-TACE treatment. The results suggest that the activity of UGTs and SULTs related to APAP metabolism in HCC patients was not affected by TACE treatment.

However, the ratio of urinary AG/AS was <14% in HCC patients, compared with normal control group. It was suggested that the metabolism pathway of APAP was changed in patient with HCC. More than 80% of APAP was metabolized into AS for HCC patients and it was metabolized mainly into AG for healthy volunteers. Maybe liver function damage was the main reason for the difference in the metabolism of APAP in HCC patients. Because the liver plays a vital role in drug metabolism, liver disease including liver cancer and chronic HBV infection can be expected to have a detrimental effect on the activity of phase I and II metabolism enzymes [16]. Previous study showed that phase I metabolism enzyme CYP1A2 activity was significantly affected by the presence of HCC disease [7]. Our study indicated that UGTs and SULTs activity was significantly affected by the presence of liver disease. The decrease or increase in UGTs and SULTs activity may lead to the toxicity and side effects of several drugs including APAP, propofol and soy isoflavone [14, 17], which were metabolized mainly by UGTs and SULTs. It was suggested that doctors should pay attention to clinical rational drug use for liver disease patients, especially the drugs, which were mainly metabolized by CYP1A2, UGTs, and SULTs.

Secondly, the environmental and hereditary factors may contribute to the individual difference in the metabolism of APAP in human body [18]. Polymorphism phenomenon of UGTs and SULTs may be related to the development of HCC disease and has some impact on the metabolism pathway of drugs including APAP, propofol, and soy isoflavone [19, 20]. The change in the metabolic pathway may be a major predisposing factor for the development of liver cancer. Therefore, further research is necessary on the relationship between polymorphism phenomenon of UGTs and SULTs and the metabolism pathway of APAP in Chinese and the relationship between polymorphism phenomenon of UGTs and SULTs, and HCC disease susceptibility.

No adverse events were seen in this study. Cigarette consumption, hepatic blood flow, and metabolism enzyme inducers and inhibitors may influence the disposition of APAP in man. These factors should be considered when interpreting the test results.

In conclusion, the metabolism of APAP is impaired in HCC patients. The plasma AS or AG to total APAP reflects that the metabolism pathway of APAP was changed in patient with HCC, and more than 80% of APAP was metabolized into AS. The APAP test in HCC patients suggests that the activity of UGTs and SULTs in HCC patients was not affected by TACE.

References

1. Lau WY, Lai EC (2009) The current role of radiofrequency ablation in the management of hepatocellular carcinoma: a systematic review. Ann Surg 249:20–25
2. Fan WJ, Zhang L, Ouyang YS, Wang LG, Wu PH (2008) Evaluating transcatheter arterial chemoembolization for primary hepatic cancer by magnetic resonance diffusion-weighted imaging. Chin Med J (Engl) 121:2353–2356
3. Caturelli E, Siena DA, Fusilli S, Villani MR, Schiavone G, Nardella M, Balzano S, Florio F (2000) Transcatheter arterial chemoembolization for hepatocellular carcinoma in patients with cirrhosis: evaluation of damage to nontumorous liver tissue-long-term prospective study. Radiology 215:123–128
4. Jeon SH, Park KS, Kim YH, Shin YS, Kang MK, Jang BK, Chung WJ, Cho KB, Hwang JS (2007) Incidence and risk factors of acute hepatic failure after transcatheter arterial chemoembolization for hepatocellular carcinoma. Kor J Gastroenterol 50:176–182
5. Xiao EH, Li JQ, Huang JF (2007) Effect of preoperative transcatheter arterial chemoembolization on proliferation of hepatocellular carcinoma cells. World J Gastroenterol 13:4509–4513
6. Lu W, Li YH, Yu ZJ, He XF, Chen Y, Zhao JB, Zhu ZY (2007) A comparative study of damage to liver function after TACE with use of low-dose versus conventional-dose of anticancer drugs in hepatocellular carcinoma. Hepatogastroenterology 54:1499–1502
7. Huang W, Qu ZQ, Li XD et al (2008) The effect of transcatheter arterial chemoembolization on CYP1A2 activity in patients with hepatocellular carcinoma. J Clin Pharm Ther 33:489–493
8. de Wildt SN, Kearns GL, Leeder JS, van den Anker JN (1999) Glucuronidation in humans. Pharmacogenetic and developmental aspects. Clin Pharmacokinet 36:439–452
9. Sharer JE, Shipley LA, Vandenbranden MR, Binkley SN, Wrighton SA (1995) Comparisons of phase I and phase II in vitro hepatic enzyme activities of human, dog, rhesus monkey, and cynomolgus monkey. Drug Metab Dispos 23:124–1231
10. Ilett KF, Ethell BT, Maggs JL, Davis TM, Batty KT, Burchell B, Binh TQ, le Thu TA, Hung NC, Pirmohamed M, Park BK, Edwards G (2002) Glucuronidation of dihydroartemisinin in vivo and by human liver microsomes and expressed UDP-glucuronosyltransferases. Drug Metab Dispos 30:1005–1012
11. Qu ZQ, Li XD, Liu HL, He P, Zhang X, Wu MC (2007) Impaired clearance of phenacetin in hepatic cirrhosis and fibrosis. Int J Clin Pharmacol Ther 45:55–62
12. Di Girolamo A, O'Neill WM, Wainer IW (1998) A validated method for the determination of paracetamol and its glucuronide and sulphate metabolites in the urine of HIV+/AIDS patients using wavelength-switching UV detection. J Pharm Biomed Anal 17:1191–1197
13. Li XD, Xia SQ, Lv Y, He P, Han J, Wu MC (2004) Conjugation metabolism of acetaminophen and bilirubin in extrahepatic tissues of rats. Life Sci 74:1307–1315
14. Takahashi H, Maruo Y, Mori A, Iwai M, Sato H, Takeuchi Y (2008) Effect of D256 N and Y483D on propofol glucuronidation by human uridine 5′-diphosphate glucuronosyltransferase (UGT1A9). Basic Clin Pharmacol Toxicol 103:131–136
15. Frati A, Salvati M, Giarnieri E, Santoro A, Rocchi G, Frati L (2002) Brain metastasis from hepatocellular carcinoma associated with hepatitis B virus. J Exp Clin Cancer Res 21:321–327

16. Huber C, Bartha B, Harpaintner R, Schröder P (2009) Metabolism of acetaminophen (paracetamol) in plants-two independent pathways result in the formation of a glutathione and a glucose conjugate. Environ Sci Pollut Res Int 16:206–213
17. Doerge DR, Chang HC, Churchwell MI, Holder CL (2000) Analysis of soy isoflavone conjugation in vitro and in human blood using liquid chromatography-mass spectrometry. Drug Metab Dispos 28:298–307
18. Mucklow JC (1988) Environmental factors affecting drug metabolism. Pharmacol Ther 36:105–117
19. Tankanitlert J, Morales NP, Howard TA, Fucharoen P, Ware RE, Fucharoen S, Chantharaksri U (2007) Effects of combined UDP-glucuronosyltransferase (UGT) 1A1*28 and 1A6*2 on paracetamol pharmacokinetics in beta-thalassemia/HbE. Pharmacology 79:97–103
20. Riches Z, Bloomer JC, Coughtrie MW (2007) Comparison of 2-aminophenol and 4-nitrophenol as in vitro probe substrates for the major human hepatic sulfotransferase, SULT1A1, demonstrates improved selectivity with 2-aminophenol. Biochem Pharmacol 74:352–358