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Activity of sulfotransferase 1A1 is dramatically upregulated in patients with hepatocellular carcinoma secondary to chronic hepatitis B virus infection

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The phase I metabolizing enzyme and phase II metabolizing enzyme play vital roles in carcinogenesis, but little is known about the changes of their activities in patients with hepatocellular carcinoma (HCC) secondary to chronic hepatitis B virus (HBV) infection. In this study phenacetin, a probe drug (1 g for men and 0.85 g for women orally), was applied for the detection of sulfotransferase 1A1 (SULT1A1) and cytochrome P4501A2 (CYP1A2) activities in 82 healthy participants and 148 HCC, 106 cirrhosis, and 41 chronic hepatitis B patients. In addition, a prospective cohort study for susceptibility to HCC was performed in 205 patients with cirrhosis secondary to chronic HBV infection. Compared with the healthy participants, SLUT1A1 activity increased by 9.7-fold in the HCC patients ($P < 0.01$). CYP1A2 activity did not significantly differ between the healthy participants and HCC patients. CYP1A2 activity decreased by 91.2% ($P < 0.01$) and 67.7% ($P < 0.05$) in the patients with cirrhosis and chronic hepatitis B, respectively; SULT1A1 activity did not increase significantly. During an approximate 2-year follow up, three of the 46 cirrhosis patients with elevated SULT1A1 activity and normal CYP1A2 activity developed HCC, but none of the 159 cirrhosis patients used as parallel controls did ($P = 0.012$). These results indicate that SLUT1A1 activity is dramatically upregulated in patients with HCC secondary to chronic HBV infection. The upregulation of SULT1A1 activity is not caused by the tumor itself. The interaction between SULT1A1 and CYP1A2 can play an important role in hepatocarcinogenesis in the Chinese population. (*Cancer Sci* 2010; 101: 412–415)

The phase I metabolizing enzyme and phase II metabolizing enzyme play vital roles in carcinogenesis and tumor response to anticancer therapy,[1–3] but little is known about the changes of their activities in patients with hepatocellular carcinoma (HCC). In previous investigations, we found that the phenacetin metabolism was different between healthy participants and HCC patients.[4] There were significant differences not only in the pharmacokinetics of phenacetin, but also in its metabolites.

Phenacetin O-deethylation is a marker reaction of cytochrome P4501A2 (CYP1A2) activity. As a probe drug, phenacetin has been extensively used to study the effects of genetic and environmental factors on CYP1A2 activity.[5–7] After the oral administration of phenacetin, its O-deethylated metabolite acetaminophen is mostly transformed into glucuronide acetaminophen or sulfate acetaminophen by hepatic UDP-glucuronosyltransferases (UGT) and sulfotransferase 1A1 (SULT1A1), then rapidly excreted by the kidney.[8,9] Therefore, the ratio of urine glucuronide acetaminophen to sulfate acetaminophen (RUGSA) can be applied to the detection of hepatic SULT1A1 activity.

Growing evidence shows that the gene–gene and gene–environment interactions involved in the metabolism of carcinogens can increase the risk of cancer, including colorectal, pancreatic, endometrial, breast, and urothelial cancer.[10–14] In the present study, we investigated the changes of SULT1A1 and CYP1A2 activities in patients with HCC secondary to chronic hepatitis B virus (HBV) infection. In addition, a prospective cohort study for susceptibility to HCC was performed in 205 patients with cirrhosis secondary to chronic HBV infection.

Materials and Methods

Participants. Eighty-two healthy participants and 148 HCC, 106 cirrhosis, and 41 chronic hepatitis B patients participated in the trial of phenacetin metabolism. Some of the participants also participated in the trial of acetaminophen metabolism. The healthy participants were workers at our hospitals. They were healthy, as shown by history, physical examinations, and laboratory tests. None consumed alcohol or smoked. No drug was taken within 2 weeks before the tests. The patients were randomly selected from the inpatients and outpatients at our hospitals from January 2006 to December 2008. The diagnostic criteria of chronic liver disease were based according to conventional clinical, serological, genetic, and virological criteria, as recognized by the International Hepatology Informatics Group.[15] The diagnosis of HCC was based on histological or cytological findings or on the presence of liver tumor with a serum α-fetoprotein value exceeding 250 ng/mL. The patients were excluded if they had been treated previously for HCC or had extrahepatic metastasis, heart or kidney dysfunction, a smoking habit, or if they were medicated with CYP1A2/SULF1A1 inducers or inhibitors within 2 weeks before the tests. The characteristics of the participants are shown in Table 1.

A prospective cohort study for susceptibility to HCC was performed in 205 patients with cirrhosis secondary to chronic HBV infection from February 2007 to December 2008. The patients were randomly selected from the inpatients and outpatients at our hospitals, including 71 of the 106 patients with cirrhosis mentioned earlier. They all had the liver function for Child–Pugh score A, the average age was 44.7 ± 13.5 years, there were 167 males compared to 38 females. The diagnosis of liver

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Table 1. Baseline characteristics of participants ($\bar{x}$ ± SD)

	Healthy participants (n = 82)	Hepatocellular carcinoma (n = 148)	Cirrhosis (n = 106)	Chronic hepatitis B (n = 41)
Age (years)	42.7 ± 9.4	48.5 ± 14.2	46.3 ± 11.6	39.6 ± 10.1
Sex (female/male)	34/48	17/131	21/85	14/27
Body weight (kg)	67.2 ± 10.4	60.7 ± 11.3	58.5 ± 12.6	63.4 ± 10.8
Child–Pugh class	—	A (125), B (23)	A (79), B (27)	A
Tumor nodes	—	≥3 (68) and <3 (80)	—	—
Maximum tumor node	—	≥3 cm (83) and <3 cm (65)	—	—

cirrhosis was according to the criteria developed by the International Hepatology Informatics Group.[15]

The study was conducted in accordance with the ethical standards set forth in the Declaration of Helsinki. Written, informed consent was obtained from each participant. The protocol was approved by the Ethics Committees of the Eastern Hepatobiliary Surgery Hospital, Renji Hospital, and the Ninth People's Hospital affiliated to the School of Medicine, Shanghai Jiaotong University.

Procedures. All of the participants were required to fast overnight. Phenacetin (1 g for men and 0.85 g for women, Jiuzhou Pharmaceutical, Shanghai, China) was ingested with 200 mL water in the morning. The blood sample was drawn at 2 h after the oral administration of phenacetin. The 24-h urine was collected after the oral administration of phenacetin. The levels of phenacetin and its metabolites (free acetaminophen, glucuronide acetaminophen, and sulfate acetaminophen) in the plasma and urine were measured by HPLC.[8] After this, 18 healthy participants (10 men, six women) and 23 HCC (18 men, five women) and 21 cirrhosis (15 men, six women) patients underwent the acetaminophen metabolism trial. These participants were randomly selected from those mentioned earlier and were required to fast overnight. Acetaminophen (1 g, Jiuzhou Pharmaceutical) was ingested with 200 mL water in the morning. At 2 h after oral administration of acetaminophen, the blood sample was drawn. The urine was collected 24 h after the oral administration of acetaminophen. The levels of acetaminophen and its metabolites (glucuronide acetaminophen and sulfate acetaminophen) in the plasma and urine were measured by HPLC.[8] The interval between the two tests was 7–9 days. The ratio of plasma total acetaminophen to phenacetin (RPTAP) was applied for the detection of CYP1A2 activity.[4] RUGSA was applied for the detection of SULT1A1 activity.

The 205 cirrhosis patients, participating in the prospective cohort study for susceptibility to HCC, underwent the phenacetin test first. RPTAP was applied for the detection of CYP1A2 activity. RUGSA was applied for the detection of SULT1A1 activity. According to RPTAP ≥0.75 and RUGSA <0.28 (the lower limits of the 95% confidence interval calculated from the healthy participants), 46 of the 205 patients (39 men, seven women) had elevated SULT1A1 activity and normal CYP1A2 activity. The 46 patients were assigned to Group A. The remainder (128 men, 31 women), who served as the parallel controls, was assigned to Group B. During the follow up, these patients underwent routine treatments and examinations, including liver ultrasound and serum α-fetoprotein every 3 months. They were reviewed on a regular basis in the clinic and contacted by telephone, email, or through regular mail. The study would end when any patient serving as a parallel control developed HCC or when HCC incidence had a statistically significant difference between the two groups.

Statistics. Data were expressed as mean ± SD. All continuous variables were tested for normality by the Kolmogorov–Smirnov test. Statistical comparison between groups was performed using the non-parametric Mann–Whitney U-test, Fisher's exact test, or Dunnett's t-test. The multivariate linear regression analysis was performed to determine whether the patients' clinical or laboratory variables independently influenced RUGSA and RPTAP. The SAS (SAS Institute, Cary, NC, USA) software package was used in the analysis. A P-value ≤0.05 was accepted as the level of significance.

Results

Metabolism of phenacetin. Compared with the healthy participants, the plasma phenacetin level increased by 2.1-fold and 54.3% in the patients with cirrhosis and chronic hepatitis B ($P < 0.01$ and $P < 0.05$). The plasma total acetaminophen level decreased by 53.2% and 26.8% ($P < 0.01$ and $P < 0.05$). RPTAP was 8.8% and 32.3% of the control value ($P < 0.01$ and $P < 0.05$), respectively. The levels of plasma phenacetin and its metabolites did not significantly differ between the healthy participants and the patients with HCC, but the recovery of urine sulfate acetaminophen was obviously increased in the patients with HCC; RUGSA was only 9.3% of the control value

Table 2. Content of phenacetin and its metabolites in plasma and urine ($\bar{x}$ ± SD)

	Hepatocellular carcinoma (n = 148)	Chronic hepatitis B (n = 41)	Cirrhosis (n = 106)	Healthy participants (n = 82)
Plasma phenacetin (μg/mL)	3.88 ± 2.67††,‡	5.62 ± 1.79*	7.93 ± 2.41**,‡‡	3.64 ± 2.56
Plasma-free acetaminophen (μg/mL)	4.74 ± 2.01††	3.80 ± 1.21*	2.44 ± 1.46**,‡‡	5.06 ± 1.83
Plasma-conjugated acetaminophen (μg/mL)	3.90 ± 2.11††,‡	2.42 ± 1.50	1.35 ± 1.06**,‡‡	3.44 ± 1.65
Plasma total acetaminophen (μg/mL)	8.64 ± 3.34††,‡	6.22 ± 2.17*	3.79 ± 2.18**,‡‡	8.50 ± 3.33
Ratio of plasma total acetaminophen to phenacetin	3.44 ± 2.92††,‡	1.90 ± 1.74*	0.52 ± 0.35**,‡‡	5.88 ± 6.59
Urine acetaminophen (%)	7.30 ± 6.12	7.05 ± 7.23	11.67 ± 11.83	6.80 ± 1.92
Urine glucuronide acetaminophen (%)	14.28 ± 3.89**,††,‡‡	54.42 ± 21.25	47.19 ± 18.80	58.02 ± 14.52
Urine sulfate acetaminophen (%)	78.42 ± 3.64**,††,‡‡	38.53 ± 17.40	41.14 ± 16.32	35.18 ± 8.75
Ratio of glucuronide acetaminophen: sulfate acetaminophen	0.18 ± 0.05**,††,‡‡	1.78 ± 0.97	1.55 ± 1.03	1.93 ± 0.54

*$P < 0.05$, **$P < 0.01$ *vs* healthy participants; †$P < 0.05$, ††$P < 0.01$ *vs* cirrhosis patients; ‡$P < 0.05$, ‡‡$P < 0.01$ *vs* chronic hepatitis B patients.

($P < 0.01$). These results are shown in Table 2. The multivariate analysis showed no influence of sex, age, baric index, HBV load, hepatitis B e-antigen status, biochemical makers of liver function, tumor size or numbers on the RUGSA and RPTAP.

Metabolism of acetaminophen. Compared with the healthy participants, the recovery of urine sulfate acetaminophen increased by 1.9-fold in the patients with HCC ($P < 0.01$). RUGSA was 14.2% of the control value ($P < 0.01$). In the patients with cirrhosis, the recovery of urine sulfate acetaminophen and RUGSA did not differ significantly from that of the healthy participants. The results are shown in Table 3.

Prospective cohort study for susceptibility to HCC. Three of the 46 cirrhosis patients with elevated SULT1A1 activity and normal CYP1A2 activity developed HCC during an approximate 2-year follow up; one (male, 51 years) in the 13th month, one (male, 46 years) in the 21st month, and another (male, 56 years) in the 23rd month. None of 159 cirrhosis patients who served as parallel controls developed HCC (Fisher's exact test, $P = 0.012$). Four patients who served as parallel controls died of liver failure during the follow up.

Discussion

Sulfation is an important pathway in phase II reactions, mediated by sulfotransferases (SULT). The molecular expression and activity of hepatic SULT have been extensively studied in humans. SULT is a gene superfamily, and four SULT families have recently been characterized; Family 1 consists of at least eight isozymes. Acetaminophen is a substrate of SULT1A1.

In the present study, phenacetin and acetaminophen were used as the probe drugs to expose the changes of SULT1A1 and CYP1A2 activities in patients with HCC secondary to chronic HBV infection. We found that the SLUT1A1 activity was dramatically upregulated in HCC patients, but the CYP1A2 activity did not reduce. These changes of SULT1A1 and CYP1A2 activities were not compensatory reactions for the impaired liver functions, because the CYP1A2 activity significantly decreased in the patients with cirrhosis or chronic hepatitis B, and SULT1A1 activity did not increase.

To verify whether the tumor itself affects the SULT1A1 activity or whether the high SULT1A1 activity makes the patients susceptible to HCC, a prospective cohort study was performed in 205 patients with cirrhosis secondary to chronic HBV infection. During an approximate 2-year follow up, three of the 46 cirrhosis patients with elevated SULT1A1 activity and normal CYP1A2 activity developed HCC, but none of 159 cirrhosis patients who served as parallel controls did. The result indicates that the upregulation of SULT1A1 activity is not caused by the tumor itself. These individuals, whose SULT1A1 activity was upregulated and CYP1A2 activity did not reduce after chronic HBV infection, are at high risk for the development of HCC. The interaction between SULT1A1 and CYP1A2 can play an important role in hepatocarcinogenesis in the Chinese population.

Zhang *et al.* recently investigated the effects of transcatheter arterial chemoembolization (TACE) on UGT and SULT activities in patients with HCC secondary to chronic HBV infection.[16] They found that TACE evidently damaged liver function, but did not affect UGT and SULT activities. The metabolism pathway of acetaminophen was altered for HCC patients. After the oral administration of acetaminophen, acetaminophen was metabolized mainly into sulfate acetaminophen in HCC patients, but mainly into glucuronide acetaminophen in the healthy controls. The ratio of urinary glucuronide acetaminophen to sulfate acetaminophen was just 13.2% of control value in HCC patients. The finding is consistent with ours.

An increasing body of evidence suggests that SULT1A1 and CYP1A2 are also involved in the metabolic activation of several carcinogens.[17–20] This is consistent with Miller's findings, whose early work on the model carcinogen 2-acetylaminofluorene (2-AAF) plus other aromatic amines led to the hypothesis that most chemicals require metabolism before being mutagenic or carcinogenic.[21] Interestingly, the first step in the activation of 2-AAF is CYP1A2-mediated *N*-hydroxylation to *N*-hydroxy-2-acetylaminofluorene, which is a substrate for SULT1A1.[22]

The interaction between drug-metabolizing enzymes and active transporters is an emerging concept in pharmacokinetics, that is, the multidrug resistance of anticancer drugs.[23,24] CYP1A2 is located on the hepatocellular microsome, as well as UGT, while SULT1A1 is a cytosolic enzyme. It is unclear whether the transporting of phenacetin metabolite acetaminophen from the microsome to cytoplasm is enhanced in HCC patients. After the oral administration of acetaminophen, the acetaminophen sulfated in the hepatocellular cytoplasm is from the blood and is not transported from the microsome to the cytoplasm. Therefore, the acetaminophen metabolism test was performed in HCC patients. We found that RUGSA did not differ significantly between the oral administration of acetaminophen and phenacetin. This indicates that a low value of RUGSA is due to the increase of SULT1A1 activity in HCC patients.

The sulfation of acetaminophen is a high-affinity and low-capacity conjugation pathway in rats and humans. In contrast, the glucuronidation of acetaminophen is a low-affinity but high-capacity conjugation pathway.[9,25] Thus, glucuronide acetaminophen is the principal metabolite in urine after the oral administration of acetaminophen. In comparison with the healthy participants, the recovery of urine total acetaminophen (including free acetaminophen, sulfate acetaminophen, and glucuronide acetaminophen) was not reduced in HCC patients; sulfate acetaminophen was obviously increased. These results indicate that the change of RUGSA does not result from the decrease in UGT activity in HCC patients.

Table 3. Content of acetaminophen and its metabolites in plasma and urine ($\bar{x}$ ± SD)

	Hepatocellular carcinoma (n = 23)	Cirrhosis (n = 21)	Healthy participants (n = 18)
Plasma-free acetaminophen (μg/mL)	3.75 ± 0.88	4.18 ± 1.16	4.07 ± 0.81
Plasma-conjugated acetaminophen (μg/mL)	4.93 ± 2.38	4.20 ± 2.88	4.58 ± 2.02
Plasma total acetaminophen (μg/mL)	8.68 ± 2.56	8.38 ± 3.28	8.65 ± 2.19
Urine acetaminophen (%)	3.81 ± 1.36**	7.42 ± 1.60*	4.86 ± 0.62
Urine glucuronide acetaminophen (%)	17.12 ± 3.37*,**	54.04 ± 6.93	53.56 ± 13.78
Urine sulfate acetaminophen (%)	79.07 ± 3.68*,**	38.54 ± 7.14	41.58 ± 13.64
Ratio of glucuronide acetaminophen: sulfate acetaminophen	0.22 ± 0.04*,**	1.46 ± 0.52	1.55 ± 1.01
Recovery of urine total acetaminophen	69.19 ± 18.51	65.24 ± 22.43	72.46 ± 23.83

*$P < 0.01$ *vs* healthy patients; **$P < 0.01$ *vs* cirrhosis patients.

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The prospective cohort study for susceptibility to HCC was not of sufficient size or duration to evaluate long-term clinical outcomes. It remains unknown why SULT1A1 activity is dramatically upregulated in patients with HCC secondary to chronic HBV infection. Further research, including the analysis of SULT1A1 and CYP1A2 polymorphisms, is necessary.

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