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Down-regulation of carboxylesterases 1 and 2 plays an important role in prodrug metabolism in immunological liver injury rats

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ABSTRACT

Liver plays a central role in xenobiotics metabolism, thus affecting the *in vivo* disposition and therapeutic effects of drugs. Carboxylesterases (CESs), with the main isoforms CES1 and CES2, are important in the metabolism of ester-type prodrugs. However, influences of immunological liver injury on the activity of CES remain undefined. In the present study, we demonstrated treatment with lipopolysaccharide (LPS) suppressed the activities of CES1 and CES2. The decreased activities of CES1 and CES2 were preliminarily assessed by the hydrolysis assay for their common substrate *p*-nitrophenyl acetate (PNPA) with rat hepatic microsomal enzyme. Subsequently, RT-PCR results showed that the levels of CES1 mRNA and mRNA of CES2 (AB010635) and CES2 (AY034877) in the model group were significantly lower than those of the normal control group ($P < 0.05$). Western blot results showed that the expressions of CES1 and CES2 proteins were decreased ($P < 0.05$). To further clarify the effects of LPS on the metabolic activities of CESs, pharmacokinetic studies were performed in rats by utilizing imidapril and irinotecan (CPT-11) as the specific substrates for CES1 and CES2, respectively. After treatment with LPS, $AUC_{0-\infty}$ and C_{max} of imidaprilat were decreased from 2084.86 ± 340.66 ng · h^{-1} · mL^{-1} and 234.66 ± 68.85 ng · mL^{-1} to 983.87 ± 315.34 ng · h^{-1} · mL^{-1} and 113.1 ± 19.69 ng · mL^{-1} ($P < 0.05$), respectively. Moreover, $AUC_{0-\infty}$ and C_{max} of SN-38 were decreased from 8100 ± 918.6 ng · h^{-1} · mL^{-1} and 144.67 ± 20.28 ng · mL^{-1} to 3270 ± 500.5 ng · h^{-1} · mL^{-1} and 56.19 ± 10.38 ng · mL^{-1} ($P < 0.05$), respectively. In summary, immunological liver injury remarkably attenuated the expressions and metabolic activities of CES1 and CES2, which may be associated with the regulatory effects of cytokines under inflammation.

1. Introduction

Liver represents the richest source of drug-metabolizing enzymes in terms of abundance and diversity, thereby playing a determinant role in the *in vivo* metabolism and clearance of drugs, endogenous compounds and xenobiotics [1]. Although many factors may alter the hepatic capacity of drug metabolism, changes in the activities of metabolic enzymes contributes the most to the alteration [2]. Chalasani et al. found that both hepatic availability and intestinal availability of midazolam were significantly higher in cirrhotic patients than those in healthy volunteers, which suggested that hepatic and intestinal CYP3A activities were reduced due to cirrhosis [3]. However, the effect of liver malfunction on hepatic metabolism is complex and depends on tissue type and the specific isoform of P-450. CYP1A and CYP3A levels and related enzyme activities are usually reduced under cirrhosis, yet CYP2C, CYP2A, and CYP2B remain almost unaltered [4]. Therefore, studying the changes of enzyme activities in hepatic injury is essential for evaluating the metabolism and therapeutic effects of drugs *in vivo*.

Carboxylesterases (CESs, EC.3.1.1.1) are members of the α, β-hydrolase family and they show ubiquitous tissue expression profiles with the maximum carboxylesterases activities in liver microsomes in many mammals [5]. Human carboxylesterases comprises a multigene family, and CES1A and CES2 families are the two major isoforms of carboxylesterases. CES1A is predominantly expressed in the liver and the lung, whereas CES2 is expressed in the gastrointestinal tract and the liver[6]. Human CES1 and CES2 govern the pharmacokinetic behaviors of most ester-containing drugs, pesticides, and prodrugs such as angiotensin-converting enzyme inhibitors (temocapril, cilazapril and imidapril) [7], anticancer drugs (CPT-11 and capecitabine) [8,9] and narcotics (cocaine, heroin and meperidine) [10]. The activity changes of CES1 and CES2 may attenuate the drug efficacy. In addition, the substrates metabolized by CES1 and CES2 differ distinctly. The compounds with small terminal hydroxyl groups and large terminal acyl groups (e.g. imidapril) are mainly metabolized by CES1, and those with large terminal hydroxyl groups and small terminal acyl groups (e.g. CPT-11)

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are primarily metabolized by CES2[11–13]. As a result, imidapril and CPT-11 are often used as the specific substrates of CES1 and CES2, respectively.

Immunological liver injury is induced by viral infections, autoimmune diseases, and xenobiotics-activated autoimmune response or specific immune response. The consequences of uncontrolled inflammatory immune responses have been illustrated during the pathogenesis of these various liver diseases [14]. Particularly, induction of gram-negative cell wall components may give rise to systemic inflammatory liver injury. The hepatocellular damage in the immunological liver injury model induced by Bacillus Calmette-Guerin (BCG) and lipopolysaccharide (LPS) is an ideal hepatotoxic model that resembles the pathological course of chronic hepatic diseases in humans [15].

So far, there were hardly any systematic studies assessing the expressions and activities of CESs under liver damage have been performed or published. Herein, we evaluated the mRNA and protein expressions of CES1 and CES2 by establishing a rat model with immunological liver injury. Besides, the pharmacokinetic changes of ester-type prodrugs were investigated by using imidapril and CPT-11 as the specific substrates for CES1 and CES2, respectively.

2. Materials and methods

2.1. Animal treatment

Male SD rats (7 weeks old, 200–220 g) were obtained from the Experiment Animal Center of Tongji Medical College, Huazhong University of Science and Technology (Wuhan, China) and housed under controlled conditions. They were maintained on a 12:12 h light–dark cycle at 21 °C. Food and water were available *ad libitum*. All procedures complied with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

2.2. Chemicals

Lipopolysaccharide (LPS) was purchased from Sigma and Bacillus Calmette-Guerin (BCG) was purchased from Wuhan Institute of Biological Products Co., Ltd. Reverse transcription kit was purchased from Invitrogen Corporation (USA). Trizol reagent and bovine serum albumin were purchased from Invitrogen Life Technologies and Gene Corporation, respectively. Imidapril hydrochloride, imidaprilat, CPT-11, SN-38 and *p*-nitrophenyl acetate (PNPA) were purchased from Toronto Research Chemicals (North York, Canada). Internal standards cetirizine and camptothecine were purchased from Haosheng Pharmaceutical Co., Ltd. (Jiangsu, China).

2.3. Animal model establishment and CES activity determination

Immunological liver injury was induced through injection of LPS into BCG-pretreated rats, as reported previously [16]. The rats were intraperitoneally injected with 8 mg BCG for seven consecutive days, and were given 5 mg · kg^{-1} LPS 3 h after the last BCG administration.The control groups receive sham injections. Then they were anesthetized by diethyl ether at 3 h, 6 h, 9 h, 12 h and 24 h and executed. The liver tissues were collected and stored in a −80 °C refrigerator. The frozen livers were thawed in 3 volumes of homogenization buffer which consisted of 50 mM Tris–HCl, 150 mM KCl and 2 mM EDTA, and then homogenized. The homogenates were centrifuged at 10,000 × g for 20 min at 4 °C. To acquire preliminary information of the activities of CESs, the S9 fractions of livers were assayed for the hydrolysis of PNPA according to the methods described previously [17].

2.4. Quantitative real-time PCR

Total RNA was extracted from liver tissue using TRIzol reagent (Invitrogen, USA). Then, the extracts were treated with DNaseI(Takara Shuzo, Japan) to prevent contamination with genomic DNA. Subsequently, first-strand cDNA was synthesized from 1 μg of each RNA by real-time PCR beads with an oligo (dT) primer (Amersham Bioscience, USA). PCR was performed (95 °C for 15 s, 58 °C for 15 s, and 72 °C for 45 s, 40 cycles) to detect the gene expression by using a set of primers: glyceraldehydes-phosphate dehydrogenase (GAPDH), sense, 5′-CGTT GACATCCGTAAAGACCT-C-3′, anti-sense, 5′-TAGGAGCCAGGGCAGTAA TCT-3′; CES1, sense, 5′-AGGTCCTGGGGAAGTATGCC-3′, anti-sense, 5′-TGCATCTTGGGAGCACATAGG-3′; CES2, sense, 5′-GGAGTGGTGTGAGA GATGCG-3′, anti-sense, 5′-CAGGTTAGAGCCCTCACGG-3′. The mRNA levels were normalized according to the level of GAPDH. Fold-differences in gene expression were calculated by the ΔΔCt equation: $\Delta CT = CT_{\text{target gene}} - CT_{GAPDH}$, $\Delta\Delta CT = \Delta CT_{\text{experiment}} - \Delta CT_{\text{control}}$, fold difference = $2^{-\Delta\Delta CT}$.

2.5. Western Blot analysis

All samples were adjusted to equal protein content before analysis and then separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). After non-specific binding sites were blocked with 5% non-fat milk, the blots were incubated with an antibody against CES1, CES2 or GAPDH. The primary antibodies were subsequently localized with goat anti-rat IgG conjugated with horseradish peroxidase. After secondary antibody incubation, the proteins were visualized using a chemiluminescnet protein detection system (Immune-Star; Bio-Rad, USA). Immunoreactive bands were detected by a gel analysis 155 system (Elurochem 5500, Alpha Innotech, USA).

2.6. Determination of imidaprilat

The plasma samples were treated with acetonitrile precipitation. The Diamonsil C18 (5 μm, 150 mm × 2.1 mm) was used as the analytical column with acetonitrile-0.1% (v/v) formic acid (1:2, v/v) as the mobile phase at a flow rate of 0.3 mL/min. The samples were ionized by electrospray ionization source in a triple-quadrupole tandem mass spectrometer, and the plasma imidaprilat and internal standard were determined with a multiple reaction monitoring mode of m/z378.1 → 206.1 and m/z389.2 → 201.1, respectively. The linearity was confirmed in the concentration range 1–500 ng/mL for imidaprilat in rat plasma. Interday and intraday coefficients of variation of the different quality control samples were both less than 10%.

2.7. Determination of CPT-11 and SN-38

Protein in plasma was precipitated with 7% perchloric acid-acetonitrile (50:50) after acidification with 7% perchloric perchloric acid. CPT-11 and SN-38 were separated on the Hypersil ODS C18 (4.6 mm × 250 mm, 5 μm) column. The mobile phase was 0.05 mol/L disodium hydrogen phosphate-methanol (50:50, v/v) containing 0.25‰ triethylamine (adjusted to pH 3.0 with phosphate) and monitored at excitation and emission wavelengths of 380 nm and 550 nm, respectively. The linear ranges for CPT-11 and SN-38 were 20–1000 ng/mL and 2–500 ng/mL, respectively. Interday and intraday coefficients of variation of the quality controls were in the range of 6.3–10.5%.

2.8. Pharmacokinetic study

The rats were randomly divided into a normal control group and a model group (n = 8). The animal model of immunological liver injury was established according to the above method. The pharmacokinetic studies were conducted 12 h after LPS injection. For imidapril analysis, rats were intragastrically administered with 10 mg · kg^{-1} imidapril, and their bloods (0.3 ml) were collected from the post-glomus venous plexus at 0.5 h, l h, 2 h, 3 h, 4 h, 5 h, 6 h, 7 h, 8 h, 10 h, 12 h and 16 h. For CPT-11 analysis, rats were injected with 20 mg · kg^{-1} CPT-11 through tail veins, and their bloods (0.3 ml) were collected from the