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Short communication

Sulfotransferase-catalyzed biotransformation of liguzinediol and comparison of its metabolism in different species using UFLC-QTOF-MS

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ABSTRACT

Liguzinediol (2,5-dihydroxymethyl-3,6-dimethylpyrazine, LZDO) is a potential agent for the low-risk treatment of heart failure. 2-*N*-acetylcysteine-LZDO (2-NAC-LZDO) and 2-cysteine-LZDO (2-Cys-LZDO) are major LZDO metabolites found in the pharmacokinetic studies of rats and beagle dogs. To elucidate the biotransformation pathway and related enzymes, an incubation system with 3′-phosphoadenosine-5′-phosphosulfate (PAPS) as a cofactor and *N*-acetylcysteine (NAC) as a trapping agent was established using liver cytosol. An ultra-flow liquid chromatography coupled with quadrupole-time-of-flight mass spectrometry (UFLC-QTOF-MS) method was used to identify the major metabolites. 2-NAC-LZDO could be detected among four species (humans, monkeys, dogs, and rats) and is the dominant metabolite in human liver cytosol (HLC). The sulfotransferase (SULT) inhibitors 2,6-dichloro-4-nitrophenol (DCNP) and quercetin at a concentration of 1 μM, suppressed 2-NAC-LZDO formation in HLC by 87 and 46%, respectively. This result suggested that sulfotransferase was involved in 2-NAC-LZDO formation. The metabolism of LZDO in different species indicated that SULT activity in dogs, rats, and monkeys was higher than that in humans. Further SULT phenotyping revealed that SULT1A1 is the predominant enzyme involved in the sulfation of LZDO. The underlying mechanism for the biotransformation of LZDO was demonstrated. The potential pathway is *via* the sulfation of LZDO to form sulfate, and the spontaneous cleavage of the sulfate group to generate highly reactive electrophilic cations, which can bind to NAC to form the major metabolites.

1. Introduction

Liguzinediol (2,5-dihydroxymethyl-3,6-dimethylpyrazine, LZDO, shown in Fig. 1A) is a water-soluble monomeric compound which has been identified as a promising candidate drug for cardiovascular diseases. Cardiac drugs widely used in the market have a narrow therapeutic window and easily induce cardiac arrhythmia. LZDO has obvious advantages and no severe adverse effects have been found [1–5].

Previously, pharmacokinetic studies in rats and beagle dogs showed that liguzinediol exhibited favorable oral bioavailability. The study of excretion and disposal of LZDO in rats and dogs found that there were multiple LZDO metabolites in urine, bile, and feces. Oxidation products, glucuronide conjugates, 2-*N*-acetylcysteine-LZDO (2-NAC-LZDO), and 2-cysteine-LZDO (2-Cys-LZDO) were detected and identified. 2-glutathione-LZDO (2-GSH-LZDO) and LZDO sulfate were found in rat bile and dog urine, respectively [6–8]. 2-NAC-LZDO and 2-Cys-LZDO (structures shown in Fig. 1, B and C) are characteristic metabolites; little is known concerning their metabolic pathway in human tissues and the underlying mechanisms of their metabolism remain unclear. It is highly important to establish an *in vitro* incubation system to generate the metabolites and a LC-MS/MS approach to detect and characterize the metabolites in both experimental animals and humans [9]. Evaluation of the enzymes which are attributed to drug metabolism is also essential.

Due to ethical constraints, a range of rodent (rats) and non-rodent (dogs and monkeys) animal species replace humans in preclinical metabolism studies of new candidate drugs. Nevertheless, extensive differences in drug metabolism between animals and humans may result

Abbreviations: LZDO, liguzinediol; Cys, cysteine; NAC, *N*-acetylcysteine; GSH, glutathione; 2-NAC-LZDO, 2-*N*-acetylcysteine-LZDO; 2-Cys-LZDO, 2-cysteine-LZDO; 2-GSH-LZDO, 2-glutathione-LZDO; HLC, human liver cytosol; HLM, human liver microsomes; RLC, rat liver cytosol; RLM, rat liver microsomes; DLC, dog liver cytosol; DLM, dog liver microsomes; MLC, monkey liver cytosol; MLM, monkey liver microsomes; SULT, sulfotransferase; DTT, dithiothreitol; PAPS, 3′-phosphoadenosine-5′-phosphosulfate; NADPH, β-nicotinamide adenine dinucleotide phosphate; DCNP, 2,6-dichloro-4-nitrophenol; PBS, phosphate-buffered saline; ANT, antipyrine; UFLC-QTOF-MS, ultra-flow liquid chromatography coupled with quadrupole-time-of-flight mass spectrometry; XIC, extracted ion chromatography

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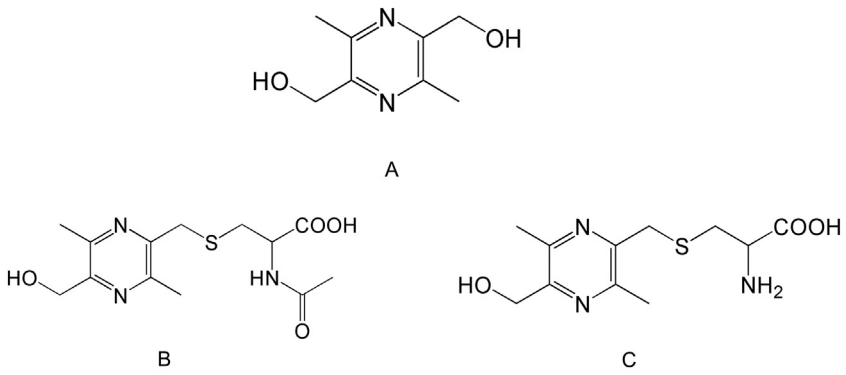

Fig. 1. Structure of LZDO (A), 2-NAC-LZDO (B), and 2-Cys-LZDO (C).

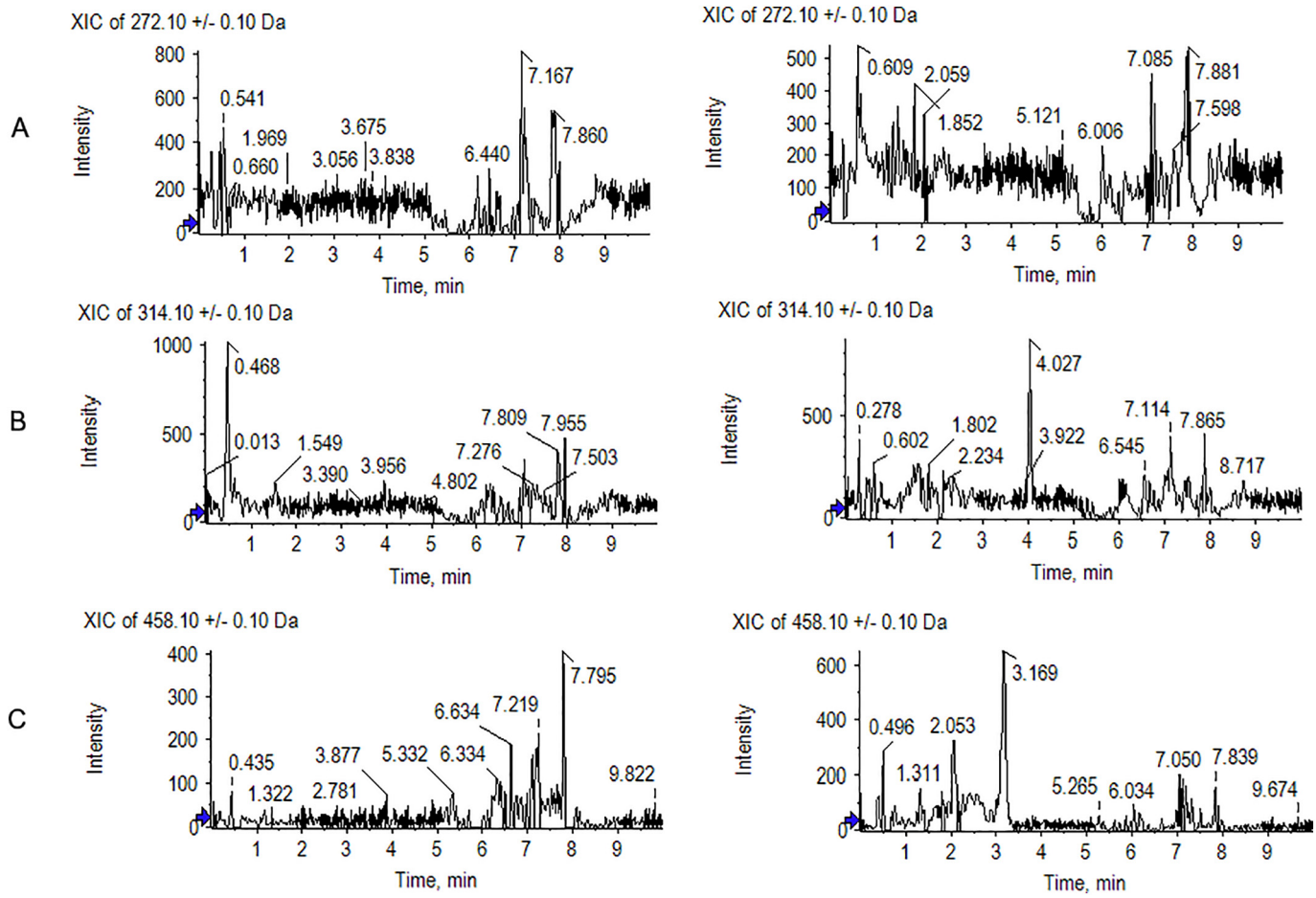

Fig. 2. Representative extracted ion chromatography (XIC) of 2-Cys-LZDO (A), 2-NAC-LZDO (B), and 2-GSH-LZDO (C) in the incubation of DLM with NADPH (left side) or PAPS (right side) and Cys, NAC, and GSH, respectively.

in species-specific effects [10–12]. Until now, the information relevant to the activity of SULT isoenzyme(s) among different species is insufficient; therefore, understanding the differences in SULT function between animals and humans is crucial [13–15].

The objectives of the present study were as follows: 1) to establish an *in vitro* incubation system and a detection method which is aimed at researching the metabolites of LZDO; 2) to compare metabolism differences between humans and other species; 3) to confirm SULT isoenzymes involved in LZDO metabolism by assays with recombinant SULT isoforms and chemical inhibition experiments.

2. Materials and methods

2.1. Chemicals and reagents

LZDO (purity > 99%, HPLC grade, confirmed by LC-MS) and 2-NAC-LZDO were kindly provided by Prof Wei LI (Nanjing University of Traditional Chinese Medicine). DTT (dithiothreitol), PAPS (3′-phosphoadenosine-5′-phosphosulfate), NADPH (β-nicotinamide adenine dinucleotide phosphate), GSH (glutathione), NAC (*N*-acetylcysteine), Cys (cysteine), and ANT (antipyrine) were purchased from Sigma-Aldrich (St. Louis, MO). Magnesium chloride was purchased from Nanjing Chemical Reagent Co. Ltd. Quercetin was purchased from Chinese pharmaceutical and Biological Products Research Institute. DCNP (2,6-