Pharmacokinetics, Pharmacodynamics and Drug Transport and Metabolism

Involvement of Rat Organic Cation Transporter 2 in the Renal Uptake of Jatrorrhizine

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ABSTRACT: Jatrorrhizine is a protoberberine alkaloid derived from *Coptis chinensis* concentrated extremely in rat kidney. In the present study, the involvement of rat organic cation transporter 2 (rOCT2) in the renal uptake of jatrorrhizine in rat was investigated through *in vitro* and *in vivo* experiments. Saturable and nonsaturable uptakes of jatrorrhizine were observed in rat kidney slices and rOCT2–Madin–Darby canine kidney (MDCK) cells. Michaelis–Menten constants of 677.8 and 21.0 μM, maximum uptake rate of 123 (pmol/min)/mg kidney and 13.7 (pmol/min)/mg protein, and nonsaturable uptake clearance of 0.054 (μL/min)/mg kidney and 0.032 (μL/min)/mg protein were observed in rat kidney slices and rOCT2–MDCK cells, respectively. As inhibitors of rOCT2, corticosterone, verapamil, and cimetidine can inhibit jatrorrhizine uptake in rat kidney slices and rOCT2–MDCK cells. Their median inhibitory concentration in rat kidney slices was 7, 78, and 538 μM, whereas that in rOCT2–MDCK cells was 1.07, 86.5, and 151.8 μM. Coadministration with 20 mg/kg corticosterone, a selective inhibitor of rOCT2, reduced the jatrorrhizine concentration in the cortex and medulla in the *in vivo* experiment. Thus, rOCT2 is mainly responsible for the renal uptake of jatrorrhizine in rat and in the regulation of jatrorrhizine concentration in the kidney. © 2012 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 102:1333–1342, 2013

Keywords: distribution; drug transport; renal transport; organic cation transporter 2; pharmacokinetics; renal uptake; jatrorrhizine; rats

INTRODUCTION

The distribution of chemicals from the systemic circulation to organs and tissues is necessary for their function and elimination. This specific distribution to some tissues is the foundation of drug targeting delivery, which features more powerful therapeutic efficacy and fewer adverse effects compared with other treatment modes. Many active ingredients and drugs in research and development have been investigated along with their characteristics of tissue distribution in animal models. Some compounds can demonstrate high levels in some tissues against a concentration gradient.[1,2] During chemical distribution to organs and tissues, the movement of chemicals across lipid

Abbreviations used: ANOVA, analysis of variance; BCA, bicinchoninic acid; CYP, cytochrome P450 enzyme; DM, diabetes mellitus; DN, diabetic nephropathy; DDI, drug–drug interactions; ESI, electrospray ionization; GFP, green fluorescence protein; HPLC, high-performance liquid chromatography; i.v., intravenous; IS, internal standard; IC_{50}, median inhibitory concentration; K_m, the Michaelis–Menten constant; LC, liquid chromatography; MDCK, Madin–Darby canine kidney cell line; MATE, multidrug and toxin extrusion; MTT, 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; MS–MS, tandem mass spectrometry; OCT2, organic cation transporter 2; P-gp, p-glycoprotein; SLC, solute carrier.

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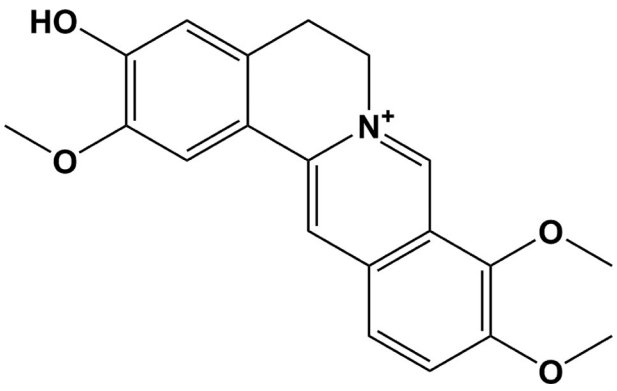

Figure 1. Chemical structure of jatrorrhizine.

bilayers is a vital premise.[3] In some cases, simple diffusion is sufficient for these chemicals to enter, as well as exit, cells while transmembrane transport is mediated by transporters using active and passive mechanisms in other instances.[3] Drug transporters can be expressed in many tissues and organs such as the intestines, brain, liver, kidney, and so on.[3,4] These transporters also play significant roles in determining the bioavailability, therapeutic efficacy, and pharmacokinetics of a variety of drugs.[4] Studies on the transporter-mediated tissue distribution of drugs can help elucidate the pharmacokinetics of drugs, their characteristics, and their role in the target organ to provide information for further research and development.

Jatrorrhizine (Fig. 1), an alkaloid derived from *Coptis chinensis* Franch, *Tinospora cordifolia*, and other medicinal plants, has a wide range of biologic activities, including antibiotic,[5] hypoglycemic,[6] antihypertensive,[7] and antiarrhythmic[8] functions. Jatrorrhizine is reportedly effective in promoting gastrointestinal motility after oral administration in rats[9,10] and can be developed as a new gastric prokinetic drug.[11] As a potential drug candidate, the pharmacokinetic characteristics of jatrorrhizine are important in drug research and development. Previous study has determined that the metabolic pathways of jatrorrhizine. Cytochrome P450 enzyme (CYP)3A1/2 and CYP2D2 are mainly responsible for demethylation, whereas uridine-5′-diphosphoglucuronosyltransferase 1A1 and 1A3 are responsible for glucuronidation in rat liver microsomes.[12] In our preliminary study, jatrorrhizine is found concentrated in rat kidney, indicating that some specific uptake transport mechanism can be responsible for its high distribution. The renal transporter of jatrorrhizine can help clarify the mechanism of its distribution and excretion through the kidney, which is significant for its further development.[13] The elucidation of the mechanism of jatrorrhizine concentration in kidney may also provide the basis for its drug–drug interaction (DDI) and potential therapeutic efficiency.

Organic cation transporters (OCTs) of the solute carrier family (SLC) 22, belonging to the organic cation transport family, have been identified as important uptake transporters that facilitate the transmembrane transport of organic cations, including several clinically used drugs.[14] The expressions of human OCT1 (SLC22A1) and OCT2 (SLC22A2) are highly restricted to the liver and kidneys, respectively.[14] OCT3 (SLC22A3) has a broad distribution.[14] Rat OCT2 is most strongly expressed in the kidney, where the OCT2 protein is localized in the basolateral membrane of proximal tubule epithelial cells. Moreover, rat OCT2 has been recognized to play an important role in the renal transport and excretion of organic cations.[4,14,15] OCTs transport various OCs of diverse molecular structures with one or two charges, which contain substrates with relative molecular masses below 500.[14] Jatrorrhizine conforms to have the above substrate characteristics. OCT2, which potentially mediates the renal uptake of jatrorrhizine, is an important renal uptake transporter and should be considered during drug discovery and development.[16] In the present study, we investigated whether OCT2 is involved in the renal uptake of jatrorrhizine and whether it regulates jatrorrhizine concentration in the kidney.

EXPERIMENTAL

Chemicals

Jatrorrhizine was purchased from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Cimetidine, verapamil, corticosterone, and phenacetin were purchased from Sigma Chemical Company (St. Louis, MO). Acetonitrile and methanol were purchased from Merck (Darmstadt, Germany). The pure water used in this study was prepared by using a MilliQ water system (Millipore, Bedford, MA). All other materials were of analytical grade or higher. A bicinchoninic acid (BCA) protein assay kit was obtained from Beyotime Institute of Biotechnology (Shanghai, China).

Animals

Sprague–Dawley rats (Grade II) of either sex, weighing 240–280 g were obtained from Experimental Animal Center of Shanghai Traditional Chinese Medicine University (Shanghai, China; Certificate No. SYXK 2007-2005). The rats were housed in an air-conditioned room at 22°C–24°C, with a 12-h dark/light cycle and free access to commercial chow and tap water. The animal experiments were performed in accordance with the Guidelines for the Care and Use