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Inhibitory effect of 1α,25-dihydroxyvitamin D_3 on excretion of JBP485 via organic anion transporters in rats

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ABSTRACT

The aim of this study was to investigate the pharmacokinetic mechanism of interaction between JBP485 and 1α,25-dihydroxyvitamin D_3 [1,25$(OH)_2D_3$]. Rats were injected intraperitoneally with 0.64 nmol/kg/day 1,25$(OH)_2D_3$ in 1 ml/kg corn oil for 5 days. The plasma and urine concentrations of JBP485 after intravenous administration and the uptake of JBP485 in kidney slices *in vitro* were determined by liquid chromatography/tandem mass spectrometry. Quantitative polymerase chain reaction, western blotting, immunohistochemical analysis and immunofluorescence were used to determine the changes in the expression of organic anion transporter (Oat)1 and Oat3 in rat kidney in response to 1,25$(OH)_2D_3$ treatment. The plasma concentrations and AUCs of JBP485 were significantly increased, while the renal clearance of JBP485 and uptake of JBP485 in kidney slices were significantly decreased after 1,25$(OH)_2D_3$ treatment. These results confirmed that 1,25$(OH)_2D_3$ inhibited renal excretion of JBP485. Moreover, 1,25$(OH)_2D_3$ decreased expression of Oat1 and Oat3 in rat kidney. Our results are novel in demonstrating an interaction between JBP485 and 1,25$(OH)_2D_3$ when they are co-administered. The mechanism of interaction between JBP485 and 1,25$(OH)_2D_3$ could be explained at least in part by inhibitory effect of 1,25$(OH)_2D_3$ on expression of Oats in rat kidney.

1. Introduction

The kidney plays an important role in the urinary excretion of drugs and their metabolites via glomerular filtration and tubular secretion (Burckhardt and Wolff, 2000; Inui et al., 2000). The first step in the tubular secretion is uptake from blood through the basolateral membrane of the epithelial cells in the proximal tubules. Transporter-mediated systems have been considered to play major roles in tubular drug uptake (Shirou et al., 2003). Organic anion transporter (Oat)1 and Oat3 are expressed on the basolateral membrane of proximal tubules, which are responsible for renal tubular active secretion to urine (Cha et al., 2001; Hasegawa et al., 2002; Lee and Kim, 2004; Nozaki et al., 2007). The substrates of Oat1 include various drugs, such as *p*-Aminohippurate (PAH), antiviral drugs, β-lactam antibiotics, anti-inflammatory agents, methotrexate and endogenous organic anions, whereas the major substrates of Oat3 include benzylpenicillin (PCG), cimetidine, estrone sulfate, pravastatin, and ochratoxin A (Deguchi et al., 2004; Uwai et al., 1998).

JBP485 (cyclo-trans-4-L-hydroxyprolyl-L-serine) is a dipeptide (Liu et al., 2000) that was first isolated from Laennec (a trade name for the hydrolysate of human placenta), and has been synthesized by chemical means (Miao et al., 2011). Animal experiments have indicated that JBP485 exhibits obvious liver (Liu et al., 1998; Wu et al., 2008; Yang et al., 2009) and gastrointestinal (Wang et al., 2011) protective effects. Our previous studies have confirmed that JBP485 is mainly excreted by Oat1 and Oat3 in rat kidneys (Zhang et al., 2010; Guo et al., 2012).

1α,25-Dihydroxyvitamin D_3 [1,25$(OH)_2D_3$], the biologically active form of vitamin D, is the natural ligand of the vitamin D receptor (VDR), has been used widely as a nutraceutical in the prevention of cancer and prolongation of longevity (Mullin and Dobs, 2007; Holick, 2004; Schwartz and Skinner, 2007; Thomas, 2006). Much is known about the molecular actions of vitamin D on the regulation of calcium and phosphorus homeostasis and its indirect feedback on parathyroid hormone (Jones et al., 1998). Activation of vitamin D requires consecutive metabolism by the liver and kidney to form 25-hydroxyvitamin D_3 and then 1α,25-dihydroxyvitamin D_3, the ligand of VDR (Feldman et al., 2005;

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Prosser and Jones, 2004). VDR is present abundantly in rat kidney where Oat1 and Oat3 are mainly expressed. Upon activation, the ligand–receptor complex recruits co-activators, heterodimerizes with the retinoid X receptor, binds to the vitamin D response element (VDRE), and regulates target gene expression (Chow et al., 2010). During the past three decades, evidence has accumulated that $1,25(OH)_2D_3$ mediates its biological activities through specific binding to the nuclear VDR.

Kidney is the VDR-rich organ. Previous studies have shown that renal mRNA and protein levels of Oat1 and mRNA level of Oat3 are decreased in a dose-dependent manner by $1,25(OH)_2D_3$ treatment. Additionally, renal mRNA levels of Pept1, Ostα, and Mrp4, and protein level of Pept1 were decreased in a dose-dependent manner (Chow et al., 2010). Our previous studies have confirmed that JBP485 is a substrate for PEPT1 and PEPT2, applying HeLa-hPEPT1 and HeLa-hPEPT2 (Guo et al., 2012). As is well known, Pept1 expressed mainly in intestine and there is low level expression of Pept1 in kidney. Pept2 is responsible for tubular reabsorption of drugs. Our previous studies have confirmed that JBP485 is mainly excreted by Oat1 and Oat3 in rat kidneys (Zhang et al., 2010; Guo et al., 2012). The cumulative urine excretion of JBP485 during 24 h was 82% (Zhang et al., 2010). Therefore, the action of Pept2 in excretion of JBP485 could be neglected.

In addition, $1,25(OH)_2D_3$ had being evaluated in clinical trials as an anti-inflammation and anti-cancer agent (Krishnan and Feldman, 2011). Therefore, it is possible for one patient suffering from hepatitis and malnutrition or inflammation to take the two drugs together. To observe whether the administration of $1,25(OH)_2D_3$ could affect the excretion of JBP485, to identify the pharmacokinetic mechanisms of interaction, and to provide a rationale for the clinical use of the drug combination, we examined the drug–drug interaction (DDI) between JBP485 and $1,25(OH)_2D_3$ using *in vivo* intravenous administration, urinary excretion and kidney slices *in vitro*, and investigated the change in mRNA and protein for Oat1 and Oat3 by quantitative polymerase chain reaction (qPCR), western blotting, immunohistochemical analysis and immunofluorescence.

Our results are believed to be the first to suggest that $1,25(OH)_2D_3$ can inhibit the renal excretion of JBP485, which is a substrate of Oat1 and Oat3. In addition, $1,25(OH)_2D_3$ inhibited renal excretion of JBP485 by decreasing expression of Oat1 and Oat3. These findings provide important information for guiding proper clinical application of JBP485 and $1,25(OH)_2D_3$.

2. Materials and methods

2.1. Chemicals

JBP485 (99.5% purity) and JBP923 (internal standard (IS), 99.5% purity) were both obtained from Japan Bioproducts Industry Co., Ltd (Tokyo, Japan). $1,25(OH)_2D_3$, PAH and PCG were obtained from Sigma–Aldrich (St Louis, MO, USA). Real-time qPCR reagents were purchased from Takara Biotechnology (Dalian, China). Polyclonal antibody and FITC-polyclonal antibody for Oat1 and Oat3 were obtained from Sigma–Aldrich. Methanol was of high-performance liquid chromatography (HPLC) grade (Tedia, Fairfield, OH, USA). Distilled water, prepared from demineralized water, was used throughout the study. All other reagents and solvents were of analytical grade and were commercially available.

2.2. Instruments

Analyses were carried out with an Agilent 1200 HPLC system consisting of a quaternary delivery system, a degasser and an auto-sampler. Isocratic chromatographic separation was done by passage through an Kromasil C18 (150 mm × 2.1 mm i.d., 5 μm; Dalian Elite Analytical Instruments Co. Ltd, China) analytical column at room temperature (22 °C). The mobile phase consisted of methanol–water (containing 0.1% formic acid) (5:95, v/v) at a flow rate of 0.5 mL min^{-1}. The run time of each sample was 2.0 min. An API 3200 triple–quadruple mass spectrometer (Applied Biosystems, Foster, City, CA,USA) was operated with an electrospray ionization (ESI) source and a Turbo IonSpray interface in positive ion mode. The optimized ionspray voltage and temperature were set at 5000 V and 600 °C, respectively. The declustering potential (DP) and entrance potential (EP) were 40 and 4 V, respectively. Multiple reaction monitoring (MRM) mode was utilized to detect the compound of interest. The selected transitions were m/z 201.2 → 86.2 for JBP485 and m/z 219.2 → 86.2 for IS, respectively, with a dwell time of 150 ms per transition. The optimized collision energy for JBP485 and IS were set at 21 and 27 V, respectively.

2.3. Animals

Male Wistar rats (weighing 250–280 g) were obtained from the Experimental Animal Center in the Dalian Medical University (permit number SCXK 2008-0002). They were allowed free access to water and were fed a chow diet. Before the pharmacokinetic experiments, animals were fasted for 12 h with water available ad libitum. The animals were sacrificed after all the experiments. All the animal experiments were conducted in accordance with the National Institutes of Health guidelines for the care and use of laboratory animals.

2.4. Induction study of $1,25(OH)_2D_3$ in rats in vivo

The $1,25(OH)_2D_3$, in anhydrous ethanol solution, was analyzed spectrophotometrically at 265 nm (UV-1700, Shimadzu Scientific Instruments, Columbia, MD, USA) and diluted in corn oil (Sigma–Aldrich) for injection. Male Wistar rats were injected intraperitoneally with 0.64 nmol/kg/day $1,25(OH)_2D_3$ in 1 ml/kg corn oil once daily for 5 days. Then the rats were applied in the following experiments on the sixth day, 24 h after the last treatment.

2.5. Plasma concentration and renal excretion of JBP485

Twenty-four hours after the last $1,25(OH)_2D_3$ treatment, the animals were anesthetized with ether for 480 min (Zhang et al., 2010). Both sides of the jugular vein were catheterized to obtain samples and to administer test compounds. A single bolus of JBP485 (6.25 mg/kg, aqueous solution) was administered intravenously via the jugular vein. Blood samples were collected at 1, 5, 10, 30, 60, 120, 240, 360 and 480 min. The bladder was cannulated with polyethylene tubing; the distal end of which flowed into an Eppendorf tube resting on a small pad of ice. Urine was collected directly from the bladder at 1, 2, 4, 6, 8, 10, 12 and 24 h. The plasma and urine concentrations of JBP485 were measured. The cumulative urinary excretion of each group was calculated. The main pharmacokinetic parameters were calculated using the 3P97 program (Zhang et al., 2010). A two-compartmental data analysis was performed for each individual JBP485 concentration-time profile. The quality of the fit was judged by evaluating the standard error of parameter estimates and the coefficient of determination (data were weighted as $1/C^2$ and $r^2 = 0.9950$).

2.6. Uptake of JBP485, PAH and PCG in kidney slices in vitro

Twenty-four hours after the last $1,25(OH)_2D_3$ treatment, rat kidneys were removed after anesthesia with ether (Zhang et al., 2010). Kidneys were decapsulated, incised and immediately placed into oxygenated Krebs-bicarbonate slicing buffer at 4 °C. Kidney slices,