Inhibitory Effect of Valsartan on the Intestinal Absorption and Renal Excretion of Bestatin in Rats

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ABSTRACT: Peptidomimetic drugs have favorable bioavailability owing to H^+/peptide transporter 1 (PEPT1) located in the intestine. Sartans are commonly used and likely coadministered with peptidomimetic drugs in the clinic; however, *in vivo* interactions between sartans and peptidomimetic drugs have not been systemically understood. Herein, the effect and mechanism of sartans on the intestinal absorption and renal excretion of the dipeptide-like drug bestatin were investigated. Following oral combination with valsartan, the plasma concentration and area under the plasma concentration–time curve of bestatin in rats decreased significantly. Bestatin absorption in rat-everted intestinal sacs was dramatically reduced by valsartan. Sartans exhibited concentration-dependent inhibition on the uptake of bestatin in human PEPT1 (hPEPT1)-HeLa cells. The cumulative urinary excretion and renal clearance of the two drugs in rats decreased after intravenous coadministration. Moreover, decreased uptake of the two drugs was observed in rats' kidney slices and human organic anion transporter (hOAT)1/hOAT3-transfected cells when coadministered. The results suggest that the intestinal absorption and renal excretion of bestatin in rats were inhibited by coadministered valsartan. Interestingly, the half-maximal inhibitory concentration (IC50) values of valsartan for PEPT1 and OAT1/3 were comparable to the theoretically estimated local drug concentration and the clinical unbound concentration, respectively, proposing possible drug–drug interaction in humans via PEPT1 and OAT1/3, which should be paid particular attention when bestatin and valsartan are coadministrated clinically. © 2013 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 103:719–729, 2014

Keywords: pharamcokinetics; drug interaction; intestinal absorption; peptide transporters; renal excretion; organic anion transporters

INTRODUCTION

Transporters influence drug pharmacokinetics and pharmacodynamics (both beneficially and deleteriously) by controlling the access of drugs to various sites in the human body, particularly the site of action.[1] Intestinal uptake transporters play an important role in the absorption of orally administered drugs. The intestinal low-affinity/high-capacity peptide transporter 1 (PEPT1) recognizes di-/tripeptides and peptide-like compounds as substrates, such as angiotensin-converting enzyme inhibitors (ACEIs) and β-lactam antibiotics.[2] Moreover, PEPT1 is now an attractive target in the design of prodrugs such as valacyclovir to improve oral (per os, p.o.) drug absorption.[3] On the contrary, concomitant intake of cephalexin or lisinopril decreases the bioavailability and cellular uptake of JBP485 (a dipeptide with antihepatitis and liver-protective effects [4]) through competitive inhibition of intestinal PEPT1 in rats and human PEPT1 expressing cells, respectively.[2,5] Renal excretion seems to be the major elimination pathway of the aforementioned peptidomimetic drugs. Furthermore, uptake transporters in the basolateral membrane are major determinants in the renal disposition of the substrate drugs.[6,7] It was previously reported that the inhibition of organic anion transporters (OATs) by JBP485, a substrate of OAT1/3, decreased the renal clearance (CL_R) of acyclovir and entecavir.[8,9] Therefore, it is reasonable to consider the underlying mechanism of transporter-based drug interactions during the course of drug development.

Bestatin is a dipeptide analog, and is commonly used clinically as an immunomodulatory and antitumor agent.[10] The intestinal absorption and tubular reabsorption of bestatin in rats are attributed to PEPT1 and PEPT2.[11] Moreover, bestatin could be accumulated in the kidney by basolateral OATs in rats[12], and be secreted into urine by a H^+/organic cation antiport system located on the brush border membrane.[13] It has been reported that the therapeutic effect of bestatin is affected by the alternative exposure mediated by drug transporters.[14,15]

Valsartan, as well as telmisartan, irbesartan, and eprosartana, are angiotensin II AT1-receptor blockers (ARB) for the treatment of hypertension. Although some sartans sterically resemble dipeptide derivatives, they are not recognized by PEPTs as substrates. However, sartans strongly inhibit hPEPT1-mediated uptake of dipeptides and cefadroxil *in vitro*.[16] In addition, serum uric acid levels can be altered by valsartan, with inhibition of renal uric acid transporters such as OATs and multidrug resistance-associated protein 4 (MRP4) as the potential underlying mechanism.[17] Furthermore, drug–drug interactions (DDIs) between valsartan and likely concomitant drugs, such as furosemide, cyclosporine, and hydrochlorothiazide, have been found in the clinic.[18–20]

Abbreviations used: DDIs, drug–drug interactions; PEPT, H^+/peptide transporter; OAT, organic anion transporter; OCT, organic cation transporter; ACEI, angiotensin-converting enzyme inhibitor; ARBs, angiotensin II AT1-receptor antagonists; MRP, multidrug resistance-associated protein; AUC, area under the plasma concentration–time curve; $t_{1/2}$, half-life; TEA, tetraethyl ammonium; KRB, Krebs–Ringer buffer; LC–MS/MS, liquid chromatography–tandem mass spectrometry; P-gp, P-glycoprotein.

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To the best of our knowledge, however, *in vivo* DDIs between peptidomimetic drugs and sartans have not been investigated systemically, despite the fact that peptidomimetic drugs (ACEI, cephalosporin, etc.) are likely used in patients receiving sartans. The purpose of this study was to explore the effect of sartans on the absorption and elimination of bestatin. *In vivo* rat pharmacokinetic studies, *in vitro* transport studies using rat-everted small-intestinal sac preparations, and *in vitro* uptake studies using rats' kidney slices were conducted to investigate changes in pharmacokinetics when bestatin and valsartan are concomitantly administered in rats. Human PEPT1 and OAT1/3 expressing cells are used to evaluate and predict the possible DDI in humans. Our results demonstrated that the intestinal absorption and renal excretion of bestatin in rats decreased significantly with concomitant valsartan administration, and that possible DDI in humans may occur via PEPT1 and OAT1/3. These findings provide useful information regarding the coadministration of dipeptide drugs and sartans.

MATERIALS AND METHODS

Materials

Bestatin was provided by Shenzhen Main Luck Pharmaceuticals Inc.,Shenzhen, People's Republic of China. Valsartan and irbesartan were obtained from Lunan Pharmaceutical Group Company, Ltd., Linyi, People's Republic of China. Telmisartan and cilostazol (internal standard) were provided by Zhejiang Kinglyuan Pharmaceutical Company, Ltd., Shangyu, People's Republic of China. Eprosartan was purchased from Dalian Meilun Medical Science and Technology Company Ltd.,Dalian, People's Republic of China. JBP485 was provided by Japan Bioproducts Industry Company Ltd. (Tokyo, Japan). Tetraethyl ammonium (TEA) and probenecid were purchased from Hubei Saibo Chemical Company, Ltd.,Wuhan, People's Republic of China. All other chemicals were of analytical grade and available from commercial sources.

Cell Culture

Mock- and hPEPT1-HeLa cells and mock- and hOAT1/3-HEK 293 cells were routinely maintained in Dulbecco's modified Eagle's medium (Invitrogen) supplemented with 10% fetal bovine serum (heat inactivated), 1% nonessential amino acid solution, 100 U/mL penicillin, and 0.1 mg/mL streptomycin and kept at 37°C in a 95% relative humidity atmosphere containing 5% CO_2.

Animals

Male Wistar rats (6–8 weeks of age, 220–250 g), obtained from the Experimental Animal Center of Dalian Medical University (Dalian, People's Republic of China; permit number SCXK 2008–0002), were used in pharmacokinetic studies. All animal experiments were performed according to local institutional guidelines for the care and use of laboratory animals. In all cases, rats were fasted overnight, allowed free access to water prior to pharmacokinetic experiments, and were anesthetized with pentobarbital (60 mg/kg, intraperitoneal).

Pharmacokinetic Interaction in Rats

Rats were divided randomly into three groups: (1) bestatin (4 mg/kg), (2) valsartan (10 mg/kg), and (3) bestatin (4 mg/kg) + valsartan (10 mg/kg). The doses used in *in vivo* pharmacokinetic studies were set according to therapeutic doses of bestatin and valsartan commonly used clinically and in previous research.[12,21] Doses used in subsequent *in vitro* studies were set near the corresponding maximum plasma concentrations to maximize the possibility for demonstrating an interaction.[17,22–25]

In absorption studies, rats received a p.o. administration of bestatin (4 mg/kg) and/or valsartan (10 mg/kg) suspended in 0.5% sodium carboxymethyl cellulose solution from a gavage needle. In renal excretion studies, bladders were cannulated with polyethylene tubing for urine collection. Rats then received an intravenous (i.v.) administration of bestatin (4 mg/kg) and/or valsartan (10 mg/kg) dissolved in 50% propylene glycol via the jugular vein.

At 1, 5, 10, 20, 30, 60, 120, 240, 360, 480, 600, and 720 min after administration, blood samples (0.1 mL) were collected via the jugular vein in heparin tubes and then centrifuged at 1000 *g* for 10 min to obtain plasma. Urine was collected directly from the bladder cannula at 1, 2, 4, 6, 8, 10, 12, and 24 h following administration. Plasma and urine samples were stored at –20°C until analytical determination of bestatin and valsartan as described below.

In Vitro Everted Intestinal Sac Preparation

In vitro everted intestinal sacs were prepared as previously described.[2] The everted intestinal sac was filled with 1 mL KRB (Krebs–Ringer buffer, containing 0.5 mM $MgCl_2$, 4.5 mM KCl, 120 mM NaCl, 0.7 mM Na_2HPO_4, 1.5 mM NaH_2PO_4, 1.2 mM $CaCl_2$, 15 mM $NaHCO_3$, 10 mM glucose, and 20 mg/L phenolsulfonphthalein as a nonabsorbable marker, pH 6.0) as a serosal solution. The distended sac was placed in 37°C oxygenated (O_2/CO_2, 95%:5%) KRB (mucosal solution) containing 10 μM bestatin and/or 5 μM valsartan. Gly–Sar (10 mM) was used as a positive control. Samples (50 μL) were collected from serosal solution at 5, 15, 30, 45, and 60 min for later bestatin and valsartan determination as described below.

In Vitro Uptake in Kidney Slices

Rats' kidney was cut into slices using a ZQP-86 tissue slicer (Zhixin Company Ltd., Xiangshan, Zhejiang, People's Republic of China; thickness 300 μm, surface area 0.15 cm^2) as previously described.[2] Slices were preincubated for 3 min in oxygenated (O_2/CO_2, 95%:5%) Krebs-bicarbonate slicing buffer (containing 120 mM NaCl, 16.2 mM KCl, 1 mM $CaCl_2$, 1.2 mM $MgSO_4$, and 10 mM NaH_2PO_4/Na_2HPO_4, pH 7.4) at 37°C. The slices were then transferred to 24-well culture plates containing 1 mL fresh buffer with bestatin (10 μM) and/or valsartan (5 μM) with gentle shaking for further incubation at 37°C or 4°C. In the inhibition assays, telmisartan (5 μM), irbesartan (5 μM), eprosartan (5 μM), JBP485 (50 μM), lisinopril (1 μM), probenecid (100 μM), and TEA (1 mM) were added to the buffer simultaneously. The uptake was terminated at the designated times by removal of the medium. Slices were immediately washed three times with 1 mL of ice-cold saline and homogenized (IKA-T 10 homogenizer; IKA, Staufen, Germany). Concentrations of bestatin and valsartan were determined as described below.

In Vitro Transporter Uptake Assays

hPEPT1-HeLa, hOAT1/3-HEK293, and corresponding mock cells were seeded in 24-well culture plates for 48 h before uptake