Prediction of Clinical Drug–Drug Interactions of Veliparib (ABT-888) with Human Renal Transporters (OAT1, OAT3, OCT2, MATE1, and MATE2K)

RYOTA KIKUCHI, YANBIN LAO, DANIEL A. J. BOW, WILLIAM J. CHIOU, MARK E. ANDRACKI, ROBERT A. CARR, RICHARD L. VOORMAN, SONIA M. DE MORAIS

Drug Metabolism and Pharmacokinetics, AbbVie Inc., North Chicago Illinois 60064

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ABSTRACT: Veliparib (ABT-888) is largely eliminated as parent drug in human urine (70% of the dose). Renal unbound clearance exceeds glomerular filtration rate, suggesting the involvement of transporter-mediated active secretion. Clinically relevant pharmacokinetic interactions in the kidney have been associated with OAT1, OAT3, OCT2, MATE1, and MATE2K. In the present study, interactions of veliparib with these transporters were investigated. Veliparib inhibited OAT1, OAT3, OCT2, MATE1, and MATE2K with IC_{50} values of 1371, 505, 3913, 69.9, and 69.5 μM, respectively. The clinical unbound maximum plasma concentration of veliparib after single oral dose of 50 mg (0.45 μM) is manyfold lower than IC_{50} values for OAT1, OAT3, OCT2, MATE1, or MATE2K. These results indicate a low potential for drug–drug interaction (DDI) with OAT1/3, OCT2, or MATE1/2K. Additional studies demonstrated that veliparib is a substrate of OCT2. In Oct1/Oct2 double-knockout mice, the plasma exposure of veliparib was increased by 1.5-fold, and the renal clearance was decreased by 1.8-fold as compared with wild-type mice, demonstrating that organic cation transporters contribute to the renal elimination *in vivo*. In summary, the *in vitro* transporter data for veliparib predicts minimal potential for an OAT1/3-, OCT2-, and MATE1/2K-mediated DDI given the clinical exposure after single oral dose of 50 mg. © 2013 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 102:4426–4432, 2013

Keywords: drug transport; drug interaction; membrane transporters; organic anion transporters; organic cation transporters; renal clearance; renal transport; veliparib

INTRODUCTION

Cancer is one of the leading causes of death in the United States, accounting for more than 20% of all deaths in 2007.[1] An extensive effort to understand the pathology of the disease and its underlying mechanisms has identified numerous novel molecular targets in potential anti-cancer therapies. Poly (ADP-ribose) polymerases (PARP) are a family of enzymes involved in multiple cellular processes such as replication, transcription, and differentiation by catalyzing the transfer of ADP-ribose units to acceptor proteins to form ADP-ribose polymers.[2] Among the 18 members of PARP family identified so far, PARP-1 and PARP-2 are involved in DNA repair via poly (ADP-ribosyl)ation of histones and DNA repair enzymes, and elevated PARP levels can result in resistance to cytotoxic chemotherapy as well as radiation. Therefore, PARP inhibitors have been considered as a potential chemotherapy and radiation sensitizers.[3–6]

Veliparib {(*R*)-2-(2-methylpyrrolidin-2-yl)-1*H*-benzo[*d*]-imidazole-4-carboxamide, ABT-888}is a novel and potent inhibitor of PARP-1 and PARP-2 with K_i values of 5.2 and 2.9 nM, respectively.[7] It is currently in clinical development for the treatment of cancer combined with DNA-damaging chemotherapeutics. Veliparib has potentiated the effects of several cytotoxic agents including temozolomide, cisplatin, cyclophosphamide, and radiation in a broad spectrum of xenografts/syngeneic tumor models.[7–9] The clinical pharmacokinetic profile of veliparib is characterized by high oral bioavailability, high volume of distribution, and primarily renal clearance. About 70% of a 50-mg dose was recovered as unchanged drug in urine.[10] Together with the area under the curve (AUC) in plasma obtained in the same study (6.01 μM × h), the renal clearance of unchanged veliparib is estimated to be 397 mL/min. Taking into account the unbound fraction in plasma (fu) of veliparib of 0.49, [11] the unbound renal clearance of veliparib (810 mL/min) is about sixfold higher than the glomerular filtration rate (GFR; 125 mL/min),[12] pointing to likely active secretion in the renal elimination of veliparib. In patients receiving veliparib, the lactam metabolite M8 was shown to be a major human plasma metabolite.[13,14]

Active tubular secretion of drugs and their metabolites occurs predominantly in the proximal tubules and involves transepithelial flux of compounds, which consists of the uptake from the blood into the epithelial cells across the basolateral membrane and subsequent efflux into the lumen across the brush border membrane. Multiple transporters are involved in this process including organic anion transporters (OATs) and organic cation transporters (OCTs) on the basolateral

Abbreviations used: PARP, poly (ADP-ribose) polymerase; fu, unbound fraction in plasma; AUC, area under the curve; GFR, glomerular filtration rate; OAT, organic anion transporter; OCT, organic cation transporter; MATE, multidrug and toxin extrusion; DDI, drug–drug interaction; PAH, para-aminohippuric acid; ES, estrone sulfate; MPP^+, 1-methyl-4-phenylpyridinium; HEK, human embryonic kidney; C_{max}, maximum plasma concentration; $C_{u,max}$, unbound maximum plasma concentration; P450, cytochrome P450 enzyme; P-gp, p-glycoprotein.

Correspondence to: Ryota Kikuchi (Telephone: +847-938-0336; Fax: +847-937-8330; E-mail: ryota.kikuchi@abbvie.com)

This paper is dedicated to Prof. Leslie Z. Benet.

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membrane, and ATP-binding cassette transporters and multidrug and toxin extrusion (MATE) transporters on the brush border membrane.[15–17] Among them, OAT1, OAT3, OCT2, MATE1, and MATE2K have been proved to be of clinical relevance in the tubular secretion of a variety of prescribed drugs and are a potential site of drug–drug interactions (DDIs) in the kidney.[18–22] Interactions with renal transporters can be beneficial or adverse. For example, coadministration of probenecid, an OAT inhibitor, decreases the renal clearance of cidofovir with a concomitant reduction in cidofovir nephrotoxicity, which is attributable to the inhibition of OAT1-mediated intracellular accumulation in renal proximal tubules.[23,24] Simultaneous use of methotrexate and nonsteroidal anti-inflammatory drugs is associated with the delay in the plasma elimination of methotrexate in patients because of the inhibition of OAT3-mediated renal uptake, and thereby with severe methotrexate toxicity.[25] OCT2 is the major transporter involved in the renal elimination of metformin, and genetic variation in this transporter plays an important role in the pharmacokinetics of metformin, thus affecting its susceptibility to OCT2 inhibition by cimetidine.[26–28] MATE1 and MATE2K are emerging transporters of interest that together with OCT2 mediate vectorial secretion of substrates into urine, and affect both pharmacokinetics and pharmacodynamics of their substrates including metformin.[29] On the basis of these clinical observations, recent guidelines proposed by the International Transporter Consortium recommend the assessment of new molecular entities as an inhibitor of OAT1, OAT3, OCT2, MATE1, and MATE2K.[22,30]

The aim of the present study was to investigate *in vitro* the interactions of veliparib and its major metabolite M8 with OAT1, OAT3, OCT2, MATE1, and MATE2K to assess the potential for transporter-mediated clinical DDIs. Furthermore, *in vitro* transporter studies and *in vivo* pharmacokinetic studies in transporter-deficient animals were conducted to determine whether these transporters are involved in the active secretion of veliparib.

EXPERIMENTAL

Materials

Para-aminohippuric acid (PAH), estrone sulfate (ES), and 1-methyl-4-phenylpyridinium (MPP^+) were used as probe substrates for OAT1, OAT3, and OCT2/MATE1/MATE2K, respectively. [^{3}H]PAH (4.53 Ci/mmol), [^{3}H]ES (54.26 Ci/mmol), and [^{3}H]MPP^+ (76.4 Ci/mmol) were purchased from PerkinElmer (Waltham, Massachusetts). [^{3}H]veliparib (3.2 Ci/mmol) was synthesized by the Department of Radiochemisty at Abbott Laboratories (Abbott Park, Illinois). The radiochemical purity of [^{3}H]veliparib was greater than 99%. All other chemicals were purchased from Sigma–Aldrich (St. Louis, Missouri) unless stated otherwise.

Uptake Experiments

Human embryonic kidney (HEK) cell lines stably expressing OAT1, OAT3, OCT2, MATE1, and MATE2K (HEK-OAT1, HEK-OAT3, HEK-OCT2, HEK-MATE1, and HEK-MATE2K) and a mock-transfected cell line (HEK-EV) were obtained from Kathleen M. Giacomini (Department of Biopharmaceutical sciences, School of Pharmacy, University of California, San Francisco). The cells were cultured in a humidified atmosphere (95%) with 5% CO_2 at 37°C in Dulbecco's modified Eagle's medium (Sigma–Aldrich) supplemented with 10% fetal bovine serum, 100 U/mL penicillin, 100 μg/mL streptomycin, and 0.1 mg/mL Hygromycin B (to maintain selection). For transport studies, cells were seeded in 24-well poly-D-lysine-coated plates ($0.9–2.5 \times 10^5$ cells/well) and cultured for 2–3 days in growth medium without Hygromycin B. The cells reached 80%–100% confluent on the day of the experiments. For uptake experiments using HEK-OAT1, HEK-OAT3, and HEK-OCT2, cells were washed three times with incubation buffer (HBSS (Hanks' Balanced Salt solution) supplemented with 10 mM HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid), pH 7.4 at 37°C). For uptake experiments using HEK-MATE1 and HEK-MATE2K, cells were preincubated with incubation buffer containing 30 mM NH_4Cl for 20 min at 37°C to generate outwardly directed proton gradient by intracellular acidification.[31] Uptake was then initiated by adding incubation buffer containing 1 μM [^{3}H]PAH, 0.1 μM [^{3}H]ES, 1 μM [^{3}H]MPP+, or 10 μM [^{3}H]veliparib in the presence or absence of multiple concentrations of known inhibitors or test compound. In the K_m/V_{max} study of OCT2-mediated uptake of veliparib, unlabeled veliparib at various concentrations (2–100 μM) was used instead of [^{3}H]veliparib. Uptake was terminated at designated times by aspirating the incubation buffer and washing the cells three times with ice-cold HBSS. To determine the uptake of radiolabeled ligands, cells were lysed in 200 μL of phosphate-buffered saline containing 0.5% Triton X-100 by shaking at room temperature for 1 h. One-hundred fifty microliter of cell lysate was transferred to scintillation vials containing 4 mL of scintillation cocktail (Insta-Gel Plus; PerkinElmer) and radioactivity associated with the cells as well as incubation buffers was determined by liquid scintillation counter (TRI-CARB 2900TR; PerkinElmer, Waltham, MA). The remaining cell lysate was filtered through a Lysate Clarification UNIFILTER (Whatman, Piscataway, New Jersey) and 10 μL of filtrate were used to determine the protein concentration by the Pierce BCA Protein Assay (Thermo Scientific, Waltham, Massachusetts) according to the manufacturer's instructions. To determine the uptake of unlabeled veliparib, veliparib associated with the cells was extracted in 500 μL of 50% acetonitrile containing 10 nM carbutamide as an internal standard, and the concentrations were quantified by liquid chromatography/tandem mass spectrometry (LC–MS/MS); veliparib was eluted from a C18 column (Fortis 2.1×30 mm^2, 5 μm) with an optimized gradient of mobile phase A, 0.1% formic acid in water, and B, 0.1% formic acid in acetonitrile, and analytes were detected in positive ion mode using multiple reaction monitoring: m/z 245.10 > 84.00. After the compound extraction, cells in each well were lysed in 300 μL of 0.1N NaOH/0.1% sodium dodecyl sulfate, and protein concentrations in the cell lysates were determined as described above. Uptake is given as the cell-to-medium concentration ratio determined as the amount of compound associated with the cells divided by the medium concentrations. Transporter-specific uptake was obtained by subtracting the uptake into mock-transfected cells from that into transporter-expressing cells. The K_m (Michaelis–Menten constant) and V_{max} (maximum uptake rate) values of probe substrates (OAT1; PAH, OAT3; ES, OCT2, MATE1, and MATE2K; MPP^+) for OAT1-, OAT3-, OCT2-, MATE1-, and MATE2K-mediated transport were 14.8 ± 0.7 μM and 979 ± 26 pmol/min/mg protein, 12.8 ± 2.5 μM and 516 ± 70 pmol/min/mg protein, 29.4 ± 4.2 μM and 2893 ± 270 pmol/min/mg protein, 45.4 ± 1.8 μM and 7625 ± 213 pmol/min/mg protein, and 37.7 ± 0.7 μM and