Evaluation of the Potency of Telaprevir and Its Metabolites as Inhibitors of Renal Organic Cation Transporters, a Potential Mechanism for the Elevation of Serum Creatinine

Tomohisa Nakada¹,†, Tomoko Kito²,†, Katsuhisa Inoue³, Satohiro Masuda⁴,††, Ken-ichi Inui⁵, Kazuo Matsubara⁶, Yoshinori Moriyama⁶, Noriko Hisanaga¹, Yasuhisa Adachi⁷, Masayuki Suzuki⁸, Ichimaro Yamada⁸ and Hiroyuki Kusuhara²,*

¹DMPK Research Laboratories Research Division, Mitsubishi Tanabe Pharma Corporation, Kisarazu, Japan
²Laboratory of Molecular Pharmacokinetics, Graduate School of Pharmaceutical Sciences, The University of Tokyo, Tokyo, Japan
³Tokyo University of Pharmacy and Life Sciences, Hachioji, Japan
⁴Department of Pharmacy, Kyoto University Hospital, Kyoto, Japan
⁵Department of Membrane Biochemistry, Okayama University Graduate School of Medicine, Dentistry, & Pharmaceutical Sciences, Okayama, Japan
⁶Research Institute, Sekisui Medical Company, Ltd., Ibaraki, Japan
⁷Development Division, Mitsubishi Tanabe Pharma Corporation, Tokyo, Japan

Summary: Telaprevir-based triple therapy is a highly effective treatment for chronic hepatitis C. However, adverse reactions include reversible and dose-dependent elevation of serum creatinine levels. We speculated that this effect reflects inhibition of the renal organic cation transporters hOCT2, hMATE1, and hMATE2-K by telaprevir or its metabolites (VRT-127394 and VRT-0922061). Telaprevir, VRT-127394, and VRT-0922061 showed negligible or weak effects on hOCT2 at concentrations of ≥20 µM, but inhibited hMATE1 by 35, 38, and 53% and hMATE2-K by 47, 45, and 61% at 100 µM, respectively. Telaprevir or its metabolites (10 µM) did not affect basal-to-apical transport of MPP⁺ across monolayers of hOCT2-hMATE1 double-transfected MDCKII cells, whereas pyrimethamine, a potent inhibitor of hMATE1, markedly inhibited MPP⁺ transport. Taken together, inhibition of hOCT2, hMATE1, and hMATE2-K is unlikely to be clinically relevant because unbound plasma concentrations of telaprevir and its metabolites reach only 2 µM following oral administration of a dose of 750 mg telaprevir. Hence, elevated serum creatinine during telaprevir therapy may not be related to direct inhibition of renal organic cation transporters.

Keywords: creatinine; MATE1; MATE2-K; OCT2; renal transporter; telaprevir

Received October 22, 2013; Accepted December 18, 2013
J-STAGE Advance Published Date: December 31, 2013, doi:10.2133/dmpk.DMPK-13-RG-118

*To whom correspondence should be addressed: Hiroyuki KUSUHARA, Ph.D., Laboratory of Molecular Pharmacokinetics, Graduate School of Pharmaceutical Sciences, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan. Tel. +81-3-5841-4774, Fax. +81-3-5841-4766, E-mail: kusuhara@mol.f.u-tokyo.ac.jp
†Tomohisa Nakada and Tomoko Kito contributed equally to this work.
‡Present address: Department of Pharmacy, Kyushu University Hospital, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan. Tomohisa Nakada, Noriko Hisanaga, Masayuki Suzuki, and Ichimaro Yamada are employees of Mitsubishi Tanabe Pharma Corporation. Yasuhisa Adachi is an employee of Sekisui Medical Co., Ltd. This study was conducted at the University of Tokyo and sponsored by Mitsubishi Tanabe Pharma Corporation.
This was reversible and telaprevir-dose dependent, with serum creatinine levels gradually returning to normal after administration of the final dose, and no elevation of serum creatinine levels after administration of 500 mg telaprevir. Therefore, it is plausible that telaprevir is associated with this adverse reaction. Creatinine is the cyclic anhydride of creatine, the end-product of skeletal muscle catabolism and is generally used as a biomarker for renal function\(^{\text{9}}\) that indicates kidney impairment.\(^{\text{7,8}}\) In a clinical study of telaprevir therapy, levels of other biomarkers for renal function, such as blood urea nitrogen, were unaltered. Therefore, the underlying mechanism of creatinine elevation may be caused by pharmacokinetic interactions of telaprevir and its metabolites that slow renal creatinine clearance.

Several clinical studies uncovered that creatinine elimination involves tubular secretion, which provides a site of interaction with drugs.\(^{\text{5}}\) To date, organic cation transporter 2 (hOCT2/SLC22A2), multidrug and toxin extrusions 1 (hMATE1/SLC47A1), and 2-K (hMATE2-K/SLC47A2) are considered to mediate directional transport of creatinine across proximal tubules.\(^{\text{10,11}}\) Some marketed drugs, such as cimetidine and pyrimethamine, are known not to affect glomerular filtration rates (GFR), as measured by renal clearance of inulin and plasma clearance of iohexol,\(^{\text{29}}\) but cause serum creatinine elevation. We previously showed that cimetidine and pyrimethamine inhibit hMATE1 and hMATE2-K, but not hOCT2, at clinically relevant doses.\(^{\text{12-14}}\) Furthermore, Imamura \textit{et al.} demonstrated that the antibacterial agent fluoroquinolone (DX-619) is a potent inhibitor of hOCT2, hMATE1, and hMATE2-K, and that inhibition of these transporters can be the mechanism underlying serum creatinine elevation during DX-619 administration in a phase 1 study.\(^{\text{15}}\) Therefore, inhibition of renal organic cation transporters by clinical doses of telaprevir may lead to reversible serum creatinine elevation. Kunze \textit{et al.} reported greater telaprevir-mediated inhibition of hOCT2 than hMATE1 using MPP\(^{+}\) and metformin as probes, respectively.\(^{\text{16}}\) Given that the maximum unbound plasma concentration of telaprevir is 2 µM, the IC\(_{50}\) of telaprevir for MPP\(^{+}\) uptake by hOCT2 (6.4 µM) may be insufficient to cause significant inhibition of hOCT2 (at most, 30% inhibition). However, two metabolites of telaprevir, VRT-127394 and VRT-0922061 (Fig. 1), have been identified in human plasma. VRT-127394 is an enantiomer of telaprevir, and the ratio of telaprevir:VRT-127394 in a phosphate buffer (pH 7.4) was found to be approximately 60:40,\(^{\text{17}}\) similar to that in human plasma. VRT-0922061 is the main metabolite found in HCV patients with maximum unbound concentrations of 2 µM after repeated oral administration of 750 mg telaprevir.\(^{\text{18}}\) Similar to drug interactions between gemfibrozil and pravastatin that cause tubular secretion,\(^{\text{19}}\) these metabolites may also contribute to inhibition of renal organic cation transporters.

The purpose of this study was to examine whether inhibition of renal organic cation transporters by telaprevir and its metabolites is clinically relevant to account for elevation in serum creatinine in Japanese HCV patients.

Materials and Methods

Materials: \(^{\text{[14C]}}\)Tetraethylammonium (TEA) (3.2 mCi/mmol) was purchased from PerkinElmer Life and Analytical Sciences (Waltham, MA). \(^{\text{[14C]}}\)Creatinine (58.6 mCi/mmol) was purchased from Moravek Biochemicals (Brea, CA), and \(^{\text{[3H]}}\)methyl-4-phenylpyridinium (MPP\(^{+}\); 80 Ci/mmol) was purchased from American Radiochemical Company (St. Louis, MO). Unlabeled TEA and pyrimethamine were purchased from Wako Pure Chemicals (Osaka, Japan), and 4-(4-(dimethylamino)styryl)-N-methylpyridinium (ASP) was purchased from Invitrogen (Carlsbad, CA). Creatinine and probenecid were purchased from Sigma-Aldrich (St. Louis, MO). All other chemicals were commercially available and were of analytical grade.

\textit{In vitro} transport study using cDNA transfectants: HEK293 cells stably expressing hMATE1 or hMATE2-K (hMATE1- and hMATE2-K-HEK293 cells, respectively) were previously established.\(^{\text{20,21}}\) HEK293 cells stably expressing reference type hOCT2 (hOCT2-HEK293) were established in Sekisui Medical Co., Ltd. (Tokyo, Japan). Cells were seeded 48 h before transport assays in poly-l-lysine- and poly-l-ornithine-coated 24-well plates at a density of 2 \times 10\(^5\) cells per well. To induce expression of transporters, cell culture medium was replaced with 5 mM sodium butyrate supplemented medium 24 h before assays. The transport study was conducted as described previously.\(^{\text{14}}\)

Uptake was initiated by the addition of substrates after cells had been washed twice and preincubated with Krebs–Henseleit buffer at 37°C for 15 min. The Krebs–Henseleit buffer contained 118 mM NaCl, 23.8 mM NaHCO\(_3\), 4.83 mM KCl, 0.96 mM KH\(_2\)PO\(_4\), 1.20 mM MgSO\(_4\), 12.5 mM HEPES, 5 mM glucose, and 1.53 mM CaCl\(_2\) and was adjusted to pH 7.4. Uptake was terminated at designated times by removing the incubation buffer and washing thrice in ice-cold Krebs–Henseleit buffer. Cells were solubilized in 0.2 N NaOH, and were then neutralized using 0.4 N HCl. Radiolabeled compounds were determined in aliquots using liquid scintillation counting. ASP concentrations were determined in aliquots using LC-MS/MS as described previously.\(^{\text{14}}\) Protein concentrations were determined using the Lowry method as described previously, with bovine serum albumin as a protein standard.\(^{\text{22}}\)
Transcellular transport study in hOCT2/hMATE1 double transfectants: Double-transfected hOCT2-hMATE1-MDCKII cells were established previously.23) Transcellular transport assays were performed as described previously using MDCKII cell monolayers grown in 24 well-transwell chambers. The incubation medium for transport experiments contained 145 mM NaCl, 3 mM KCl, 1 mM CaCl₂, 0.5 mM MgCl₂, 5 mM d-glucose, and 5 mM MES (pH 5.5–6.5) or 5 mM HEPES (pH 7.0–8.5) and pH was adjusted using NaOH or HCl.

After removal of culture medium from both sides of monolayers, 0.25 ml of incubation media was added to the apical side and 1 ml to the basolateral side, and cells were incubated for 120 min at 37°C. The incubation medium on either the apical or basolateral side was replaced with 1 ml of incubation medium containing substrates. To examine transcellular transport, 100 µl aliquots of incubation medium were periodically collected from each side, and radiolabeled compounds were determined using liquid scintillation counting.

Statistical analysis: Data are presented as the mean ± S.E. Two-tailed unpaired Student’s t-tests and a one-way analysis of variance followed by Dunnett’s post hoc test were used to identify significant differences between groups where appropriate. Differences were considered significant when p < 0.05.

Results

Inhibition of hOCT2 in cDNA-transfected cells: The specificity of uptake was confirmed by saturation or inhibition in the presence of excess TEA, which markedly reduced the uptake of all the test substrates. Effects of telaprevir and its metabolites VRT-127394 and VRT-0922061 were examined according to uptake of [¹⁴C]TEA and ASP by hOCT2-expressing HEK293 and mock-vector-transfected cells (mock; Fig. 2). Weak inhibition of TEA uptake was observed in the presence of telaprevir and VRT-127394, although the effect of telaprevir was not statistically significant and VRT-0922061 had no effect on TEA uptake. Telaprevir, VRT-127394, and VRT-0922061 had no effect on ASP uptake. The effects of telaprevir on the uptake of [¹⁴C]TEA and ASP were examined; telaprevir did not affect MPP⁺ uptake at 100 µM, but significantly inhibited creatinine uptake at 100 µM (Fig. 3). Addition of albumin to the incubation media attenuated the effect of telaprevir (data not shown).

Inhibition of hMATE1 and hMATE2-K in cDNA-transfected cells: The activities of hMATE1 and hMATE2-K were confirmed using inhibition by pyrimethamine (Fig. 4). Telaprevir, VRT-127394, and VRT-0922061 inhibited TEA uptake by hMATE1 and hMATE2-K in a concentration-dependent manner (Figs. 4A and 4B). At 100 µM, telaprevir, VRT-127394, and VRT-0922061
inhibited hMATE1- and hMATE2-K-mediated uptake of [14C]TEA by 35, 38, and 53%, and 47, 45 and 61%, respectively.

Overexpression of hMATE1 and hMATE2-K in HEK293 cells induced TEA-sensitive uptake of creatinine, which was not observed in mock-vector transfected cells (Figs. 4C and 4D). At 100 µM, telaprevir, VRT-127394, and VRT-0922061 significantly inhibited hMATE1-mediated creatinine uptake by 55, 54, and 46%, respectively, and almost completely inhibited hMATE2-K-mediated uptake.

**Effects of telaprevir on transcellular transport of MPP+ across monolayers of hOCT2/hMATE1 double-transfected cells:** hOCT2/hMATE1 double-transfected cells showed directional transport of MPP+ from basal to apical media, whereas directional transcellular transport of MPP+ was not observed in mock cells. Pyrimethamine markedly inhibited transcellular transport, indicating that hOCT2 and hMATEs were active in these cell lines. Transcellular transport of MPP+ across monolayers of double transfecants was determined in the presence of telaprevir and its metabolites (10 µM). In these experiments, telaprevir and VRT-127394 had no effect on directional transcellular transport of [3H]MPP+, although VRT-0922061 insignificantly inhibited transport by 15% (Fig. 5). Similarly, telaprevir did not inhibit bidirectional transcellular transport of [14C]TEA at 10 µM or 100 µM (Supplemental Fig. 1).

**Discussion**

In the present study, we examined whether inhibition of renal organic cation transporters by telaprevir and its metabolites leads to serum creatinine increase during telaprevir therapy in Japanese HCV patients.

After multiple administrations of telaprevir to Japanese HCV patients, the mean Cmax value for telaprevir was 5.4 µM at steady state,24) and the unbound maximum plasma concentration (Cmax,unbound) was approximately 2 µM.18) Under steady-state conditions, maximum plasma concentrations of VRT-127394 and VRT-0922061 were almost equivalent to those of telaprevir. At clinically relevant concentrations, no inhibition of hOCT2 by telaprevir or its metabolites was observed (Fig. 2). Because this interpretation follows free drug hypothesis, we also tested the possibility that albumin-bound telaprevir inhibits hOCT2. However, 500 µM albumin rather attenuated these inhibitory effects (data not shown), and thus, this possibility can be excluded. Based on the present data, inhibition of hOCT2 by telaprevir is therefore unlikely to be clinically relevant, even though we tested the contribution of its metabolites. Notably, a large discrepancy in the hOCT2 inhibition potency of telaprevir exists between this and a previous report by Kunze et al., in which telaprevir inhibited hOCT2 with an IC50 of 6.4 µM.16) Because the previous study used MPP+ as a test probe, we speculated the inhibition potency of telaprevir against OCT2 is substrate dependent. Among the four substrates tested (TEA, ASP, MPP+, and creatinine), creatinine uptake was more potently inhibited by telaprevir than MPP+ and ASP uptake; however, the effect remained weaker than that in the previous report. The reason for this discrepancy remains unknown.

Kunze et al. also revealed that telaprevir has an ability to inhibit hMATE1 with an IC50 value of 23.0 µM.16) In the present study, we...
demonstrated that telaprevir and its metabolites show similar hMATE1-inhibition potency (Fig. 4). However, in contrast to the previous report, the effect was weaker; uptake was inhibited only by <50%, even at an inhibitor concentration of 100 µM. Inhibition potency against hMATE1 was similar to that against hMATE2-K when TEA was used as a test substrate (Fig. 4). On the other hand, hMATE2-K appears to be more sensitive to telaprevir and its metabolites when creatinine is used as a test substrate, although this may lack relevance because creatinine uptake by MATE2-K is limited for quantitative evaluation. To discuss the in vivo relevance of MATE inhibition by telaprevir and its metabolites, their unbound kidney concentrations need to be estimated. After oral administration of [14C]telaprevir (10 mg/kg) to rats, the kidney-to-plasma concentration ratio was approximately 2 (data not shown), suggesting that telaprevir is not highly concentrated by active transport in the kidney. Assuming that the unbound concentration of telaprevir in the kidney is comparable to that in plasma, inhibition of hMATEs by telaprevir and its metabolites may be negligible.

Although hMATEs act as efflux transporters in vivo, we examined the effects of telaprevir on uptake from the extracellular space. To evaluate the inhibition potency against efflux by hMATE, we also examined the effect of telaprevir and its metabolites on the transcellular transport of MPP+ across monolayers of MDCK cells expressing both hOCT2 and hMATE1. The double transfectants showed directional transport of the common substrates of hOCT2 and hMATE1 across epithelial cells. Directional transport of MPP+ was shown and was totally inhibited by pyrimethamine at the concentration where it caused significant inhibition of only hMATE1, but not hOCT2 (Fig. 5). At 10 µM, telaprevir or its metabolites did not affect transcellular transport of MPP+.

Fig. 5. Time profiles of transcellular transport of MPP+ in control and hOCT2/hMATE1 double-transfected MDCKII cells
Transcellular transport of [3H]MPP+ (12 nM) across monolayers of mock (A) and hOCT2/hMATE1 double-transfected MDCKII cells (B) or hOCT2/hMATE1 double-transfected MDCKII cells in the presence of pyrimethamine (100 µM; C), telaprevir (10 µM; D), VRT-127394 (10 µM; E), or VRT-0922061 (10 µM; F). Transcellular transport in apical-to-basal (a to b, open square) and basal-to-apical (b to a, open circle) directions at apical pH of 6.0; transcellular transport in the apical-to-basal (a to b, closed square) and basal-to-apical (b to a, closed circle) directions at apical pH of 7.4. Points and vertical bars represent the mean ± S.E. (n = 3).

Copyright © 2014 by the Japanese Society for the Study of Xenobiotics (JSSX)
inhibition of the transcellular transport of TEA at 100 µM. There are two possibilities: 1) intracellular concentration of telaprevir was limited compared with the extracellular concentration, or telaprevir may show difference in its inhibition potency when it has an access to MATE1 from the extracellular or intracellular sides, and 2) transcellular transport of TEA in the double transfectants may be uptake-limited (the efflux across the apical membrane is greater than the basolateral efflux). When the transcellular transport is uptake-limited, unless the efflux via MATEs is inhibited considerably, the overall directional transport will not be affected. Taken together, these studies demonstrate no contribution of telaprevir or its metabolites to inhibition of hOCT2 or hMATEs at clinically relevant concentrations.

In conclusion, it is unlikely that telaprevir, VRT-127394, or VRT-0922061 inhibit directional transport of creatinine through hOCT2, hMATE1, or hMATE2-K at clinically relevant concentrations. VRT-127394 and VRT-0922061 are two possibilities: 1) intracellular concentration of telaprevir was uptake-limited (the efflux-limited compared with the extracellular concentration, or telaprevir may show difference in its inhibition potency when it has an access to MATE1 from the extracellular or intracellular sides, and 2) transcellular transport of TEA in the double transfectants may be uptake-limited (the efflux across the apical membrane is greater than the basolateral efflux). When the transcellular transport is uptake-limited, unless the efflux via MATEs is inhibited considerably, the overall directional transport will not be affected. Taken together, these studies demonstrate no contribution of telaprevir or its metabolites to inhibition of hOCT2 or hMATEs at clinically relevant concentrations.

Acknowledgments: We appreciate Dr. Heike Gutmann (Drug Transporters) for her generous help with the information on human OCT2-HEK cells.

References


