Short Communication

Interactions of Fluoroquinolone Antibacterials, DX-619 and Levofloxacin, with Creatinine Transport by Renal Organic Cation Transporter hOCT2

Masahiro Okuda, Naoko Kimura and Ken-ichi Inui*
Department of Pharmacy, Kyoto University Hospital, Faculty of Medicine, Kyoto University, Kyoto, Japan

Full text of this paper is available at http://www.jstage.jst.go.jp/browse/dmpk

Summary: Interactions of DX-619, a novel fluoroquinolone antibacterial, and levofloxacin (LVFX) with the human renal organic cation transporter hOCT2 were studied. The intracellular accumulation of [14C]creatinine in stable transfectants of HEK293 cells expressing hOCT2 (hOCT2-HEK293) as well as vector-transfected HEK293 cells (VEC-HEK293) was evaluated in the presence of DX-619 and LVFX at various concentrations. When added extracellularly, both DX-619 and LVFX inhibited the uptake of [14C]creatinine (5 μM) by hOCT2-HEK293 cells in a dose-dependent manner. Unlike in hOCT2-HEK293 cells, the uptake in VEC-HEK293 cells was not inhibited by either fluoroquinolone suggesting that hOCT2 was specifically involved in the inhibition. The apparent IC50 value for the inhibition of [14C]creatinine uptake in hOCT2-HEK293 cells was 1.29 ± 0.23 μM for DX-619 and 127 ± 27 μM for LVFX, indicating DX-619 to be ~100-fold more potent than LVFX at inhibiting the transport of [14C]creatinine by hOCT2. A Dixon plot revealed that the inhibition by DX-619 of the hOCT2-mediated transport of [14C]creatinine was competitive. Fluoroquinolone antibacterials have the ability to inhibit the transport of creatinine by hOCT2, with DX-619 being much more effective than LVFX.

Key words: DX-619; levofloxacin; hOCT2; creatinine; fluoroquinolone; organic cation transporter

Introduction

The level of creatinine in serum is the most commonly used clinical index of renal function. It is well recognized that the serum creatinine level is influenced by factors such as the patient’s age and sex as well as the method of its determination. Because creatinine is mostly eliminated through urine via glomerular filtration, its renal clearance is regarded to be proportional to renal function. However, significant secretion of creatinine occurs at renal tubules, and may cause an overestimation of the glomerular filtration rate (GFR) especially in patients with decreased renal function.1-3) Shemesh et al.3) reported that the tubular secretion of creatinine is relatively constant regardless of a decreased glomerular filtration rate. Attempting to obtain a better estimation of the glomerular filtration rate by using creatinine clearance, Berglund et al.,10) Burgess et al.11) and van Acker et al.6) administered trimethoprim10) and cimetidine6,11) to patients in which the tubular secretion of creatinine was blocked. Similarly, the application of cationic drugs to patients could lead to incorrect estimations of renal function because of decreased tubular secretion of creatinine.

In the renal proximal tubules, organic ion transporters mediate the tubular secretion of ionic drugs, thus contributing to the efficient extrusion of harmful substances from the body.7-11) Human organic cation transporter 2, hOCT2, is the most abundant organic cation transporter so far reported in the human kidney.12) hOCT2 should play significant roles in the basolateral translocation of some H2-receptor antagonists13,14) and biguanides15) into epithelial cells in the renal proximal tubules. hOCT2 also accepts endogenous monoamines such as norepinephrine, serotonin, histamine and dopamine.16) We found that creatinine is a specific substrate for the organic cation transporter hOCT2 expressed at the basolateral membranes of the human
kidney, but not by the hepatic organic cation transport-
er hOCT1. In addition, the transport of creatinine by hOCT2 was inhibited by cationic drugs at around their clinical concentrations.\(^{17}\) We also clarified that the tran-
sepithelial transport of creatinine across LLC-PK\(_1\) cell
monolayers was directional from the basolateral to apical side, and the characteristics of creatinine’s uptake across basolateral membranes was comparable to that
demonstrated in hOCT2-expressing HEK293 cells.\(^{18}\)

DX-619 is a novel des-fluoro(6) quinolone highly
active against gram positive bacteria.\(^{19–24}\) DX-619 has
two dissociation constants, \(pK_a1 = 6.4\) and \(pK_a2 = 8.3,\)
showing that the drug is a zwitterion in the physiological
environment. Fukuda \textit{et al.}\(^{24}\) reported that the area
under the concentration-time curve (AUC)/MIC ratio
in the lungs for DX-619 was significantly higher than
that for sitafloxacin and ciprofloxacin when tested in
mice. However, a phase 1 clinical trial of DX-619 has
not been completed yet, so very little is known about the
pharmacokinetic properties of DX-619. We have
previously clarified that levofloxacin (LVFX) is excreted
into urine \textit{via} tubular secretion in addition to glomeru-
lar filtration \textit{via} specific transport system.\(^{25–27}\) In
addition, the tubular secretion of LVFX should be
partly mediated by P-glycoprotein.\(^{28}\) Furthermore, we
clarified in cultured epithelial cells derived from pig
kidney that LVFX interacts with the organic cation
transport system at apical membranes but not at
basolateral membranes at its therapeutic concentra-
tions.\(^{29–31}\) However, no report has been made so far
regarding the interactions of fluoroquinolone antibac-
terials with hOCT2-mediated transport of creatinine.
In the present study, we investigated interactions of
DX-619 and LVFX with the hOCT2-mediated transport
of creatinine, and then referred to its clinical sig-
nificance.

\section*{Materials and Methods}

\textbf{Materials:} DX-619, (−)7-[(3R)-3-(1-aminocy-
clopropyl)pyrrolidin-1-yl]-1-[(1R,2S)-2-fluoro-1-cy-
clopropyl]1,4-dihydro-8-methoxy-4-oxoquinoline-3-
carboxylic acid, and LVFX (Fig. 1) were provided by
Daichi pharmaceuticals Co. Ltd. (Tokyo, Japan).
[2,\textsuperscript{14}C]Creatinine hydrochloride (55 mCi/mmol) was
purchased from American Radiolabeled Chemicals
(St. Louis, MO, USA). Creatinine was obtained from
Nacalai Tesque (Kyoto, Japan). 1-Methyl-4-phenyl-
pyridinium (MPP) iodide was purchased from Sigma-
Aldrich (St. Louis, MO, USA). All other compounds
used were of the highest purity available.

\textbf{Cell culture:} hOCT2-expressing HEK293 cells
(hOCT2-HEK293) and mock-transfectants established
by the transfection of the plasmid vector pCMV6-XL4
into HEK293 cells (VEC-HEK293) in our previous
study\(^{17}\) were cultured in complete medium consisting of
Dulbecco’s modified Eagle’s medium with 10% fetal
bovine serum in an atmosphere of 5% \(CO_2/95\%\) air at
37°C. For uptake experiments, the cells were seeded
onto poly-D-lysine-coated 24-well plates at a density of
2.0 × 10\(^5\) cells per well. The cell monolayers were used at
day 3 of culture for uptake experiments. In the present
study, cells were used between the 73rd and 78th pas-
sages.

\textbf{Uptake experiments using HEK293 transfectants:}
The uptake of [\textsuperscript{14}C]creatine into cells was measured
with monolayer cultures of hOCT2-HEK293 and VEC-
HEK293 cells grown on poly-D-lysine-coated 24-well
plates. The cells were preincubated with 0.2 mL
of incubation medium for 10 min at 37°C. The medium
was removed, and 0.2 mL of incubation medium
containing 5 \(\mu\)M [\textsuperscript{14}C]creatine was added. The com-
position of the incubation medium was as follows (in
mM): 145 NaCl, 3 KCl, 1 CaCl\(_2\), 0.5 MgCl\(_2\), 5 D-glucose,
and 5 HEPES (pH 7.4). The medium was aspirat-
ed off at the end of the incubation, and the monolayers
were rapidly rinsed twice with ice-cold incubation
medium. The cells were solubilized in 0.5 mL of 0.5 N
NaOH, and then the radioactivity in aliquots was
determined by liquid scintillation counting using a
Packard TRI-CARB 1900CA (PerkinElmer, Wellesley,
MA, USA). The protein content of the solubilized cells
was determined by the Bradford method using a Bio-
Rad protein assay kit (Bio-Rad Laboratories, Hercules,
CA, USA) with bovine \(\gamma\)-globulin as a standard.\(^{32}\) The
uptake of [\textsuperscript{14}C]creatine was determined in the presence
of various concentrations of DX-619 and LVFX. The
apparent IC\(_{50}\) values were calculated from inhibition
plots on the equation, \(V = V_0 /[1 + (I/IC_{50})^n]\), by a
Inhibition of hOCT2-mediated uptake of \([^{14}C]\)creatinine by various concentrations of DX-619 and LVFX: HEK293 cells transfected with hOCT2 were incubated at 37°C for 2 min with 5 \(\mu M\) \([^{14}C]\)creatinine (pH7.4) in the presence of DX-619 (in \(\mu M\): 0.1, 0.3, 1, 3, 10, 30) or LVFX (in mM: 0.01, 0.03, 0.1, 0.3, 1, 3). Each point represents the mean ± SE of three separate experiments using three monolayers. When not shown, SE is included within the symbols.

Table 1. Effect of DX-619, LVFX and MPP on the uptake of \([^{14}C]\)creatinine by VEC-HEK293 and hOCT2-HEK293 cells. The uptake of 5 \(\mu M\) \([^{14}C]\)creatinine by VEC-HEK293 and hOCT2-HEK293 cells was evaluated for 2 min in the absence (control) and presence of DX-619 (300 \(\mu M\)), LVFX (3 mM) or MPP (1 mM). Data are expressed as the mean ± SE from three separate experiments. **, \(p<0.01\) compared to the control by Dunnet’s test.

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>VEC-HEK293 (nmol/mg protein/min)</th>
<th>hOCT2-HEK293 (nmol/mg protein/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(% of control)</td>
<td>(% of control)</td>
</tr>
<tr>
<td>Control</td>
<td>2.11 ± 0.37 (100.0)</td>
<td>16.58 ± 4.50 (100.0)</td>
</tr>
<tr>
<td>DX-619 (300 (\mu M))</td>
<td>1.78 ± 0.18 (84.7)</td>
<td>1.93 ± 0.29** (11.7)</td>
</tr>
<tr>
<td>LVFX (3 mM)</td>
<td>1.61 ± 0.09 (76.4)</td>
<td>1.95 ± 0.16** (11.7)</td>
</tr>
<tr>
<td>MPP (1 mM)</td>
<td>1.60 ± 0.22 (76.0)</td>
<td>1.92 ± 0.24** (11.5)</td>
</tr>
</tbody>
</table>

Results

Inhibition of hOCT2-mediated \([^{14}C]\)creatinine uptake by various concentrations of DX-619 and LVFX: In order to assess the interaction of DX-619, a novel fluoroquinolone antibacterial, and LVFX with the hOCT2-mediated transport of creatinine, we measured the uptake of \([^{14}C]\)creatinine by hOCT2-HEK293 and VEC-HEK293 cells in the absence and presence of various concentrations of DX-619 and LVFX. The hOCT2-HEK293 cells were confirmed to be able to transport \([^{14}C]\)tetraethylammonium, a typical substrate for hOCT2, as well as \([^{14}C]\)creatinine (data not shown). As demonstrated in our previous study,17) the initial uptake rate of 5 \(\mu M\) \([^{14}C]\)creatinine was obtained over a 2-min period with hOCT2-HEK293 cells. In the present study, both DX-619 and LVFX inhibited the uptake of 5 \(\mu M\) \([^{14}C]\)creatinine for 2 min in a dose-dependent manner (Fig. 2). The IC\textsubscript{50} values for the inhibition were 1.29 ± 0.23 \(\mu M\) and 127 ± 27 \(\mu M\), respectively. Table 1 gives a summary of the uptake in the absence (control) and presence of DX-619 (300 \(\mu M\)), LVFX (3 mM) and MPP (1 mM). Unlike in hOCT2-HEK293 cells, the uptake of 5 \(\mu M\) \([^{14}C]\)creatinine in VEC-HEK293 cells was not inhibited by either of these fluoroquinolones.

Fig. 2. Inhibition of hOCT2-mediated uptake of \([^{14}C]\)creatinine by various concentrations of DX-619 and LVFX. HEK293 cells transfected with hOCT2 were incubated at 37°C for 2 min with \(5 \mu M\) \([^{14}C]\)creatinine (pH7.4) in the presence of DX-619 (in \(\mu M\): 0.1, 0.3, 1, 3, 10, 30) or LVFX (in mM: 0.01, 0.03, 0.1, 0.3, 1, 3). Each point represents the mean ± SE of three separate experiments using three monolayers. When not shown, SE is included within the symbols.

Fig. 3. Dixon plot of the inhibition of DX-619 of hOCT2-mediated transport of \([^{14}C]\)creatinine. HEK293 cells transfected with hOCT2 were incubated at 37°C for 2 min with \([^{14}C]\)creatinine (5 \(\mu M\), open circle; 10 \(\mu M\), closed circle; 15 \(\mu M\), open triangle) in the absence and presence of DX-619 (1 \(\mu M\) and 3 \(\mu M\)). Each point represents the mean ± SE for three monolayers. When not shown, SE is included within the symbols.

Dixon plot of the interaction of DX-619 with hOCT2-mediated transport of \([^{14}C]\)creatinine: Next, we made a Dixon plot to clarify the type of interaction of the fluoroquinolones with the hOCT2-mediated transport of \([^{14}C]\)creatinine. Cellular uptake of \([^{14}C]\)creatinine (5 \(\mu M\), 10 \(\mu M\) and 15 \(\mu M\)) was measured for 2 min in the absence and presence of DX-619 (1 \(\mu M\) and 3 \(\mu M\)). The Dixon plot clearly indicated that the inhibition by DX-619 of the hOCT2-mediated transport of \([^{14}C]\)creatinine was competitive (Fig. 3).

Statistical analyses: Data was analyzed statistically with Dunnett’s test. \(P\) values of less than 0.05 were considered to be significant.
Inhibition of Creatinine Transport by Fluoroquinolones

Discussion

In the present study, both DX-619 and LVFX inhibited the transport of \[^{14}\text{C}\]creatinine in hOCT2-expressing HEK293 cells. Neither fluoroquinolone had an effect in mock-transfected HEK293 cells, suggesting that DX-619 interrupted the hOCT2-mediated transport of \[^{14}\text{C}\]creatinine specifically.

Serum creatinine is the most commonly used clinical marker of kidney function, because its clearance is mostly mediated by glomerular filtration. It is generally regarded that the serum creatinine level can be raised by reducing the glomerular filtration rate in patients with decreased renal functions. However, the usefulness of serum creatinine as a marker is limited by factors such as the age, sex, and amount of muscle of the patients as well as the method by which the level of creatinine is determined. Blood urea nitrogen (BUN) is also used as a clinical marker of renal function, although its value can be affected by the amount of protein ingested, protein catabolism, bleeding in the digestive tract, and the urea synthesis rate. Verho et al.\(^3\) reported that the peak plasma concentration of LVFX was 6.4 \(\mu M\) after the oral administration of a 200-mg tablet in 6 healthy volunteers. In the present study, the peak plasma concentration was much lower than the \(\text{IC}_{50}\) of LVFX to inhibit the transport of \[^{14}\text{C}\]creatinine (127 \(\pm\) 27 \(\mu M\)), suggesting that LVFX should not inhibit creatinine transport via hOCT2 at therapeutic concentrations. Given that similar peak plasma concentrations are obtained by administration of DX-619, the drug should markedly inhibit the transport of creatinine by hOCT2, because the \(\text{IC}_{50}\) value of DX-619 to inhibit the transport of \[^{14}\text{C}\]creatinine (1.29 \(\pm\) 0.23 \(\mu M\)) was lower than the expected peak plasma concentration of DX-619. Actually, when DX-619 was administered to the healthy volunteers, an elevated serum creatinine level was observed with no apparent change in the level of BUN. In addition, the elevated serum creatinine level returned to normal with the systemic elimination of DX-619, suggesting that the tubular secretion of creatinine may be inhibited by DX-619 (unpublished observation in the clinical study).

In conclusion, DX-619 and LVFX inhibited the hOCT2-mediated transport of \[^{14}\text{C}\]creatinine in a competitive manner. These results suggest that the elevated serum creatinine levels after the administration of cationic drugs may be caused by the inhibition of tubular secretion of creatinine mediated in part by hOCT2.

Acknowledgment: This work was supported in part by a grant-in-aid for Research on Advanced Medical Technology from the Ministry of Health, Labor and Welfare of Japan, by a grant-in-aid for Scientific Research from the Ministry of Education, Science, Culture and Sports of Japan, and by the 21st Century COE program “Knowledge Information Infrastructure for Genome Science”.

References

14) Motohashi, H., Uwai, Y., Hiramoto, K., Okuda, M. and Inui, K.: Different transport properties between


