Effects of Sodium Bicarbonate and Ammonium Chloride Pre-treatments on PEPT2 (SLC15A2) Mediated Renal Clearance of Cephalexin in Healthy Subjects

Rui LIU, Audrey May Yi TANG, Yen Ling TAN, Lie Michael George LIMENTA and Edmund Jon Deoon LEE*
Department of Pharmacology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore

Summary: PEPT2 mediates the H⁺ gradient-driving reabsorption of di- and tri-peptides, and various peptidomimetic compounds in the kidney. This study examines the influence of urinary pH modification through sodium bicarbonate and ammonium chloride pre-treatments on the function of PEPT2 in healthy subjects, using cephalexin as the probe drug. Sixteen male subjects received a single oral dose of 1000 mg cephalexin under ammonium chloride and sodium bicarbonate treatment, respectively, with a wash-out period of one week. The study subjects were genotyped for PEPT2 polymorphic variants. Cephalexin concentrations in plasma and urine were determined by high performance liquid chromatography. The mean renal clearance of cephalexin was significantly higher under ammonium chloride treatment than that under sodium bicarbonate treatment (P < 0.01). This difference was significant for PEPT2*2/*2 (P = 0.017) but not for PEPT2*1/*1 (P = 0.128). No differences were observed for other pharmacokinetic parameters. The findings of this study suggest that urinary pH changes may alter the pharmacokinetics of PEPT2’s substrates. This effect was more obvious for the PEPT2*2/*2.

Keywords: Asian; cephalexin pharmacokinetics; PEPT2 (SLC15A2) pharmacogenetics

Introduction

The H⁺/peptide co-transporter 2 (PEPT2, gene SLC15A2) is found in numerous organs and actively mediates the translocation of its substrates across biological membranes driven by H⁺ gradient.1 It is highly expressed in kidney,2,3 predominantly contributing to the renal reabsorption for its substrates. PEPT2 is also expressed in peripheral nervous system,4–6 central nervous system,7–10 enteric nervous system,11 lung,12 mammary gland13,14 and heart15 with lower level. The substrates of PEPT2 include di- and tri-peptides, endogenous peptidomimetic compounds such as 5-aminolevulinic acid,16–18 and many peptidomimetic drugs including beta-lactam antibiotics, angiotensin-converting enzyme (ACE) inhibitors, the anticancer drug bestatin and antiviral drug valacyclovir.19–22 In vitro studies have demonstrated that the PEPT2 protein is affected by extracellular pH.1

Two main haplotypes of PEPT2 have been reported, PEPT2*1 and PEPT2*2.23 However, the functional difference between PEPT2*1 and PEPT2*2 is still controversial.23–25 Consequently it remains unclear how much the genetic variants of PEPT2 affect the pharmacokinetics of drug substrates of PEPT2. This may be of clinical significance as the frequencies of PEPT2*1 and PEPT2*2 vary considerably among populations.23,26

Cephalexin is a first-generation beta-lactam antibiotic prescribed for the treatment of respiratory tract, skin and soft tissue infections. In humans, after rapid and near complete absorption from the gastrointestinal tract, more than 90% of cephalexin is eliminated unchanged in the urine within 8 hours.27,28 In the kidney, cephalexin is mainly reabsorbed by PEPT2 at the apical/basolateral membrane of the renal proximal tubule cells.29 Animal and in vitro studies show that cephalexin is a high affinity PEPT2 substrate.30–32 Okumura et al. reported that the renal reabsorption fractions of cephalexin are high and range between 41–53% in healthy Japanese subjects.33,34

Received; April 27, 2010, Accepted; October 18, 2010, J-STAGE Advance Published Date: November 12, 2010
*To whom correspondence should be addressed: Professor Edmund Jon Deoon Lee, Department of Pharmacology, Yong Loo Lin School of Medicine, National University of Singapore, Block MD11, Level 5, #05-09, Clinical Research Centre, 10 Medical Drive, Singapore 117597.
Tel. +65 6516 3677, Fax. +65 6873 7690, E-mail: edmund_jd_lee@nuhs.edu.sg
Financial support for the research: This work was supported by a grant from the National Medical Research Council, Singapore (NMRC/1014/2005).
In this study, we used the specific PEPT2 substrate, cephalexin, to examine potential interactions between urinary pH and PEPT2 haplotypes. In addition, the participants of this study were Asian subjects residing in Singapore. High frequencies of PEPT2*1/*1 and PEPT2*2/*2 genotypes in Asian Indians and Chinese, respectively, have been previously reported in our population. Even though we did not specifically recruit according to ethnicity, the ethnic composition of the two genotypic groups reflected differences in genotype frequencies between the individual ethnic groups. Hence we are unable to exclude the possibility of ethnic effects for the observed differences in cephalexin pharmacokinetics in this study.

**Methods**

**Subjects:** A total of sixteen unrelated healthy male subjects participated in this study after giving written informed consent. The pre-study routine screening included medical history, physical examination, complete blood cell count, serum chemical analysis and urinalysis. Subjects were excluded if there was history or presence of renal disease that would interfere with the clearance of cephalexin, history of immunological hypersensitivity or previous adverse reactions to cephalexin and other cephalosporins, and evidence of significant laboratory abnormalities. Alcohol and tobacco were not permitted during the study period. The study protocol was approved by the Institutional Review Board of Changi General Hospital, Singapore.

**Study designs:** This was an open-label, randomized, two phase, cross-over study. After an overnight fast, each subject received a single oral dose 1,000 mg of cephalexin (coated tablet, supplied by Changi General Hospital pharmacy) given every 3 h (+2, +5 and +8 h) and 0.8 g before sleeping (−9 h) or (ii) 1.2 g of sodium bicarbonate (tablet, supplied by Changi General Hospital pharmacy) every 3 h (+2, +5 and +8 h) and 2.4 g before sleeping (−9 h). The order of treatments was open and balanced, and the subjects were randomly allocated to the treatments. Venous blood samples (10 ml each) were drawn before and after each treatment. Urine samples were collected by centrifugation. Urine samples were stored at −70°C until the time of analysis. A standardized lunch was served 4 hours after administration of cephalexin.

**Pharmacokinetic analysis of cephalexin:** Cephalexin concentrations in plasma and urine were quantified by a high-performance liquid chromatography (HPLC) as previously described with minor modification. Briefly, plasma was extracted using a solid-phase extraction method and urine was diluted with purified water, followed by chromatography on a C18 reversed-phase analytical column. The mobile phase consisted of sodium dihydrogen phosphate, pH 3.5 and acetonitrile (88:12, v/v) delivered at a flow rate of 1 ml min⁻¹. The eluent was monitored at 260 nm. The concentrations of cephalexin were determined from peak area ratios of cephalexin to the internal standard.

The lower limit of quantification was 50 ng ml⁻¹ and 5 µg ml⁻¹ in plasma and urine, respectively. The coefficients of variation for intraday and interday were less than 4.7% and the accuracy ranged from 97.1% to 103.9%.

Pharmacokinetic analysis was performed using KINETICA ver. 4.4.1 software (Thermo Fisher Scientific, Waltham, MA, USA). The maximum plasma concentration (Cₘₐₓ, µg ml⁻¹) and corresponding sampling time (tₘₐₓ, h) were identified from observed data. The area under the plasma concentration–time curve from time 0 to the last sampling time, t (AUC₀⁻₉, µg ml⁻¹ h) was calculated using the linear trapezoidal rule. The half-life (t½,h) was calculated from the elimination constant kₑ = ln 2/½ (time), where l½ is the slope of the linear terminal part of the plasma concentration versus the time curve after semilogarithmic transformation. The renal clearance from 0 to t (Clᵣ, ml min⁻¹) was calculated as AE₀⁻₉/AUC₀⁻₉, where AE₀⁻₉ is the accumulative amount of the drug excreted in urine from time 0 to t.

**PEPT2 (SLC15A2) Genotyping:** Genomic DNA was extracted from peripheral leucocytes using the AxyPrep Blood Genomic DNA Miniprep Kit (Axygen Bioscience, California, USA). Previously reported polymerase chain reaction (PCR) primers were used to amplify exon 13, 15 and 17 to determine PEPT2 haplotype. PCR amplification was carried out in a total volume of 50 µl containing 1 × Master Mix (Promega, Madison, WI, USA), 0.2 µm of each primer (Research Biolabs, Singapore) and 100 ng of DNA. Annealing temperature and cycling number were modulated with respect to each PCR fragment. PCR reactions were run on the Peltier Thermal Cycler (DNA Engine Dyad; MJ Research Inc., Waltham, MA, USA). Agarose gel electrophoresis (2%, 150 V, 35 min) was performed to verify the PCR products. Then, the PCR products were purified using Exonuclease I (New England Biolabs, Beverly, MA, USA) and Shrimp alkaline Phosphatase (Promega) and sequenced with the same PCR primers, using BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) on an ABL Prism Model 3100 Avant Genetic Analyser (Applied Biosystems, Foster City, CA, USA).
**Table 1. Effects of ammonium chloride and sodium bicarbonate pre-treatments on PEPT2 mediated pharmacokinetics of cephalexin**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Pooled (n = 15)</th>
<th>PEPT2*1/*1 (n = 7)</th>
<th>PEPT2*2/*2 (n = 8)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AC (a)</td>
<td>SB (b)</td>
<td>AC (c)</td>
<td>SB (d)</td>
</tr>
<tr>
<td>$C_{\text{max}}$ (µg ml$^{-1}$)</td>
<td>32.8 ± 8.2</td>
<td>32.6 ± 7.4</td>
<td>34.4 ± 9.1</td>
<td>32.8 ± 7.0</td>
</tr>
<tr>
<td>95% CI</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$t_{\text{max}}$ (h)</td>
<td>28.2–37.3</td>
<td>28.5–36.8</td>
<td>27.1–39.8</td>
<td>25.2–42.3</td>
</tr>
<tr>
<td>range</td>
<td>0.75–1.50</td>
<td>0.75–1.50</td>
<td>0.75–1.50</td>
<td>0.75–1.50</td>
</tr>
<tr>
<td>AUC$_{0-\infty}$ (µg ml$^{-1}$ h)</td>
<td>74.6 ± 11.2</td>
<td>73.0 ± 11.0</td>
<td>74.6 ± 11.4</td>
<td>73.6 ± 12.4</td>
</tr>
<tr>
<td>95% CI</td>
<td>68.3–80.8</td>
<td>66.9–79.0</td>
<td>64.0–85.5</td>
<td>62.1–84.7</td>
</tr>
<tr>
<td>$t_{1/2}$ (h)</td>
<td>1.00 ± 0.13</td>
<td>1.11 ± 0.18</td>
<td>1.22 ± 0.12</td>
<td>1.25 ± 0.35</td>
</tr>
<tr>
<td>95% CI</td>
<td>1.04–1.18</td>
<td>1.01–1.21</td>
<td>1.06–1.57</td>
<td>0.96–1.35</td>
</tr>
<tr>
<td>AE (mg)</td>
<td>932 ± 244</td>
<td>601 ± 120</td>
<td>1147 ± 341</td>
<td>782 ± 126</td>
</tr>
<tr>
<td>95% CI</td>
<td>617–1365</td>
<td>457–864</td>
<td>832–1463</td>
<td>665–899</td>
</tr>
<tr>
<td>$\text{CLR}$ (ml min$^{-1}$)</td>
<td>245 ± 86</td>
<td>169 ± 45</td>
<td>266 ± 95</td>
<td>183 ± 50</td>
</tr>
</tbody>
</table>

AC, ammonium chloride; SB, sodium bicarbonate; Data are given as mean ± standard deviation, except for $t_{\text{max}}$, which is given as median and range. $C_{\text{max}}$, the maximum plasma concentration; $t_{\text{max}}$, the time to reach maximum concentration; AUC, the area under the plasma concentration-time curve; $t_{1/2}$, elimination half life; CLR, the renal clearance; CI, confidence interval; n, number of individuals.

**Results**

**Subject demographics:** Fifteen subjects (age, 29.8 ± 7.0 years; weight, 72.4 ± 7.3 kg) completed the study because one subject prematurely withdrew due to the shy bladder syndrome. Among them, seven were genotyped as PEPT2*1/*1 (age, 30.3 ± 6.5 years; weight, 73.9 ± 7.2 kg) and eight subjects were PEPT2*2/*2 (age, 29.4 ± 7.4 years; weight, 70.7 ± 4.7 kg).

**Urinary pH obtained:** After ammonium chloride treatment, urinary pH decreased to 5.76 from 6.50, whereas sodium bicarbonate treatment resulted in an increase in pH from 6.23 to 7.15. pH differences between treatments up to 3 h post-dose were significant ($P < 0.05$).

**Urinary pH modification and cephalexin pharmacokinetics:** Cephalexin was not detected in samples in the last collection interval for plasma (10 h) and urine (8–12 h), so all data analyses were applicable to the time from 0 to 8 h. The effects of ammonium chloride and sodium bicarbonate treatments on cephalexin pharmacokinetics are summarized in Table 1 and Figure 1. The mean renal clearance of cephalexin was significantly higher under ammonium chloride treatment than that under sodium bicarbonate treatment ($\text{CLR}_{\text{ac}} = 245 ± 86 \text{ ml min}^{-1}$; $\text{CLR}_{\text{sb}} = 169 ± 45 \text{ ml min}^{-1}$; $P < 0.01$). No significant difference in $C_{\text{max}}$, $t_{\text{max}}$, AUC0-8 or $t_{1/2}$ was observed.

**Subgroup analysis of PEPT2*1 and PEPT2*2:** Subgroup analyses of PEPT2*1/*1 and PEPT2*2/*2 are summarized in Table 1 and Figure 1. The mean renal clearance of cephalexin was significantly higher under ammonium chloride treatment than that under sodium bicarbonate treatment in PEPT2*2/*2 carriers ($\text{CLR}_{\text{ac}} = 228 ± 79 \text{ ml min}^{-1}$; $\text{CLR}_{\text{sb}} = 156 ± 39 \text{ ml min}^{-1}$; $P = 0.017$) but was not statistically significantly different ($P = 0.128$) in PEPT2*1/*1 subjects ($\text{CLR}_{\text{ac}} = 266 ± 95 \text{ ml min}^{-1}$; $\text{CLR}_{\text{sb}} = 206 ± 86 \text{ ml min}^{-1}$).

Copyright © 2011 by the Japanese Society for the Study of Xenobiotics (JSSX)
monium chloride and sodium bicarbonate treatments
PEPT2*1/*1
between pH differences were signiﬁcantly different. The aqueous solubility of cephalxin is stable under the urine pH occasions obtained in this study.61 In addition, there was no significant differences in urine volume or GFR between ammonium chloride and sodium bicarbonate treatments or between PEPT2*1/*1 and PEPT2*2/*2 genotypic group.

Discussion

In the present study, we evaluated the effects of sodium bicarbonate and ammonium chloride pre-treatments on the renal clearance of cephalxin in healthy subjects. Although the pre-treatment regiments were relatively mild, urinary pH differences were signiﬁcantly different. The results of this study showed that the renal clearance of cephalxin is potentially affected by urinary pH, implying the functional variability of PEPT2 under these conditions. This finding is especially so for subjects with the PEPT2*2/*2 genotype.

The urine volume, GFR and cephalxin pharmacokinetics in this study were comparable to those previously reported.74–55 The aqueous solubility of cephalxin is stable under the urine pH occasions obtained in this study.61 In addition, there was no signiﬁcant differences in urine volume or GFR between ammonium chloride and sodium bicarbonate treatments or between PEPT2*1/*1 and PEPT2*2/*2 genotypic group.

Previous in vitro studies showed that the function of PEPT2 is markedly affected by the extracellular pH but the effects of the extracellular pH on the function of PEPT2 are dependent on the net charge of the substrates.49–50 Generally, ion-coupled transport systems are activated by their respective coupling ions. In most cases, this is due to the ability of the ions to increase the affinity of the transport systems for their substrates.50 This has been documented in a previous in vitro study done in human PEPT2-transfected HeLa cells, which showed the optimum pH for the afﬁnity to the neutral Gly-Sar to be from 6.0 to 7.0.51 Similar results were obtained in rabbit PepT2-transfected Xenopus oocytes; when the extracellular pH was reduced from 8.0 to 6.0, the uptake of neutral D-Phe-Ala by PEPT2 increased almost 15-fold. PEPT2 activity showed no pronounced reduction by reducing pH further to 5.5.50 However, in PEPT2-transfected SKPT cells, H⁺ influenced only the Vmax of uptake and not the apparent afﬁnity of the cationic dipeptide Alanine-Lysine (Ala-Lys) and the anionic dipeptide Alanine-Aspartate (Ala-Asp). It was inferred that H⁺ stimulates PEPT2 primarily by increasing the maximal velocity of the transporters with no detectable influence on the substrate afﬁnity of cationic or anionic dipeptides.53 The ﬁndings from previous in vitro studies about the inﬂuence of extracellular pH on the uptake of beta-lactams by PEPT2 are conﬂicting. Ganapathy et al. found the optimal pH for cephalxin was 6.0 in rPepT2-transfected SKPT cells59 while Boll M. et al. observed the uptake of cefadroxil by rPepT2 was highest at pH 6.5 in rPepT2-transfected Xenopus oocytes.21 The results of this study imply that the optimal pH for the afﬁnity of cephalxin to human PEPT2 is likely to be from acidic to neutral (6.23 to 7.15; sodium bicarbonate treatment group), consistent with in vitro studies using the neutral dipeptide Gly-Sar and Phe-Ala in PEPT2-transfected HeLa cells but not with the study by Ganapathy et al. The reason for this discrepancy is uncertain but may be due to different cellular systems used in these studies.

Cephalxin is actively taken up by intestinal epithelial cells via another H⁺/peptide co-transporter - PEPT1.1,19,31,53–56 In rats, PEPT1 has been shown responsible for about 50% of total cephalxin intestinal absorption.57 The pre-treatments of the two urinary pH modiﬁers in this study, ammonium chloride and sodium bicarbonate treatments,
may also alter the gastrointestinal pH and hence the function of PEPT1 to some extent. It is possible that the observed pharmacokinetic differences may have been confounded by the concurrent effects in both the absorption and elimination phases. In this regard, a slight delay in absorption following sodium bicarbonate treatment compared to ammonium chloride was observed although there was no significant difference as seen from the concentration–time profile plots.

The two main PEPT2 haplotypes, namely PEPT2*1 and PEPT2*2, include the three most abundant non-synonymous SNPs that affect the following amino acids: F350L, S409P, and L509R. The PEPT2*1 protein contains the amino acids Leu350, Pro409, and Arg509 while the PEPT2*2 protein contains amino acids Phe350, Ser409, and Lys509. More recent studies further exploring the functional variability between PEPT2*1 and PEPT2*2 have shown controversial results as indicated in the uptake of one standard dipeptide substrate - glycyl-sarcosine (Gly-Sar). Pinsonneault et al. observed that PEPT2*1 displayed significantly 3-fold lower \( K_m \) values but similar \( V_{max} \) for Gly-Sar compared with PEPT2*2 when expressed in Chinese hamster ovary cells. They also found that PEPT2*1 and PEPT2*2 showed different sensitivity to extracellular pH. For PEPT2*1, the highest uptake was seen at pH 6.0, whereas for PEPT2*2 uptake at pH 6.0 was significantly low. At the other extracellular pH values investigated, no difference in uptake was observed. In contrast, Sala-Rabanal et al. found that \( K_m \) values for Gly-Sar were up to 2-fold higher in PEPT2*1 than in PEPT2*2 transfected Xenopus oocytes while the \( V_{max} \) values of both variants were similar at the extracellular pH 5.0. Moreover, in a study using primary human lung epithelial cells generated from donors with different PEPT2 genotypes and grown in two-chamber Transwell system (apical side, pH 6.5; basolateral side, pH 7.9), Bahadduri et al. revealed different results; homozygous PEPT2*1/*1 and PEPT2*2/*2 carriers were similar in \( K_m \) and \( V_{max} \) values for Gly-Sar but heterozygous PEPT2*1/*2 carriers were significantly lower than both the homozygous. In the present study, PEPT2*2/*2 genotypic subgroup showed higher sensitivity to urinary pH modifier treatment than PEPT2*1/*1 genotypic subgroup but there were no significant differences in the pharmacokinetic parameters between these two genotypic groups observed from the urinary pH range 5.76 to 7.15. The results of this study appear to be functionally consistent with the previous findings by Bahadduri et al. but in apparent contrast to the studies by Pinsonneault et al. and Sala-Rabanal et al.

The findings of this study show that pre-treatment with sodium bicarbonate and ammonium chloride may alter the pharmacokinetics of PEPT2 substrates such as cephalixin. This effect was more obvious for PEPT2*2/*2 carriers. While these effects are related to urinary pH, we are unable to exclude other effects on intestinal absorption. Further studies are required particularly across a wider variety of substrates.

References


34) Shen, H., Smith, D. E., Yang, T., Huang, Y. G., Schnermann, J. B. and Brosius, F. C., 3rd: Localization of PEPT1 and PEPT2

35) Copyright © 2011 by the Japanese Society for the Study of Xenobiotics (JSSX)


