RESEARCH ARTICLE

Investigation of the role of organic cation transporter 2 (OCT2) in the renal transport of guanfacine, a selective α2A-adrenoceptor agonist

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Abstract

1. Guanfacine is a selective α2A-adrenoceptor agonist primarily excreted as its unchanged form through urine in human. This study was to investigate the involvement of organic cation transporter 2 (OCT2) in the renal tubular secretion of guanfacine.

2. Transport of guanfacine was characterized using human embryonic kidney (HEK293) cells expressing human OCT2 (hOCT2). The inhibitory effect of cimetidine on guanfacine uptake was also examined. In addition, in vivo pharmacokinetic study was conducted in rats to assess the effects of cimetidine on the pharmacokinetics of guanfacine.

3. The accumulation of guanfacine in hOCT2-transfected HEK293 cells was both time- and concentration-dependent, and markedly higher than that in mock cells. The apparent Km and Vmax values of guanfacine uptake by hOCT2 were 96.19 ± 7.49 μM and 13.03 ± 0.49 nmol/mg protein/min, respectively. Guanfacine transport mediated by hOCT2 was significantly inhibited by a typical OCT2 inhibitor cimetidine with an IC50 value of 93.82 ± 1.13 μM. Co-administration of cimetidine significantly decreased the plasma clearance (CLp) as well as the renal clearance (CLR) of guanfacine in rats in a dose-dependent manner, resulting in a noticeable increase in the systemic exposure of guanfacine.

4. These results indicated that OCT2 may be involved in the renal disposition of guanfacine.

Introduction

Guanfacine, a selective α2A-adrenergic receptor agonist, is a guanidine compound widely used to treat mild-to-moderate hypertension (Safar et al., 1982; Sorkin & Heel, 1986; Van Zwieten et al., 1984). In recent years, extended-release formulation of guanfacine has been approved for the treatment of attention-deficit hyperactivity disorder (ADHD) symptoms in children and adolescents including hyperactivity, impulsivity and inattention because of its effective enhancement of prefrontal cortex function by activating post-synaptic α2A-adrenoceptors (Arnsten, 2010; Biederman et al., 2008; Cruz, 2010; Li et al., 2013). Early publications have reported that in healthy individuals, guanfacine is well absorbed orally with high absolute bioavailability and is excreted primarily into urine with approximately 30–75% of the dose as its unchanged form (Cruz, 2010; Kiechel, 1980; Sorkin & Heel, 1986). Additionally, the cumulative urinary excretion and renal clearance of guanfacine were much higher in human with normal renal function than that in patients with renal failure, demonstrating the importance of kidney in the elimination of guanfacine. The renal clearance of guanfacine was also much greater than the glomerular filtration rate (GFR), suggesting the contribution of an active secretory process to its renal excretion (Carchman et al., 1987; Kiechel, 1980; Kirch et al., 1980). Although early in vivo pharmacokinetic studies have indicated the existence of active tubular secretion of guanfacine, the molecular mechanism underlying the renal transport of guanfacine has not been clarified yet.

Kidney is the major elimination organ for a wide variety of drugs and their metabolic wastes through both glomerular filtration and tubular secretion. In particular, tubular secretion is an active transport process involving various kinds of membrane transporter proteins, such as organic anion transporters (OATs) and organic cation transporters (OCTs) (Burckhardt, 2012; Koepsell, 2013). Membrane transporters are localized in tubular cells, which are the potential site of drug–drug interactions resulting in possible changes in the urinary excretion and systemic drug concentrations. The significance of OCTs in the renal disposition of drugs has been suggested by an increasing number of literatures. Among OCTs, OCT2 is predominantly expressed in the basolateral side of proximal tubule cells. It has been considered as a critical contributor in the renal tubular secretion of numerous...
therapeutic agents and xenobiotics characterized as relatively hydrophilic, small molecular mass organic cations or weak bases that are positively charged at physiological pH; therefore, facilitating the first step of renal elimination (International Transporter Consortium et al., 2010; Koepsell et al., 2007; Morrissey et al., 2013; Nies et al., 2011).

Guanfacine is a hydrophilic, weak alkaline compound with small molecular mass (Kiechel, 1980). Also, it shares similar chemical structure with those guanidine compounds that have already been identified as substrates for OCT2, such as metformin, phenformin, guanidine, creatinine and amino-guanidine (Kimura et al., 2005, 2009; Sogame et al., 2013). Given the fact that guanfacine is primarily excreted through kidney and its chemical structure fits in well with the substrate characteristics of OCT2, it is highly possible that OCT2 may be involved in the renal secretion of guanfacine.

The current study aimed at clarifying the possible role of OCT2 in the renal transport of guanfacine. We investigated the effects of the model OCT2 inhibitor cimetidine on guanfacine with the integration of in vitro experiments using hOCT2-expressing HEK293 cells and in vivo pharmacokinetic studies in rats. To the best of our knowledge, this is the first study illuminating the potential relationship between guanfacine and renal OCT2.

Materials and methods

Materials

Guanfacine hydrochloride (99.9% purity) was kindly provided by Henan Zhongshuai Medical Technology Development Co. Ltd (Zhengzhou, China). Cimetidine injection (100 mg/mL) was purchased from Jiangsu Pengyao Medical Technology Development Co. Ltd (Yixing, China). Tetraethylammonium bromide (98.0% purity), cimetidine (99.8% purity) and enalaprilat (99% purity, used as internal standard) were obtained from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Methanol and acetonitrile were of HPLC grade and purchased from Tedia Company Inc. (Fairfield, OH). All other reagents were of analytical grade.

Uptake experiments using hOCT2-expressing system

HEK293 cells stably expressing hOCT2 and plasmid vectors alone were constructed as previously described (Gorboulev et al., 1997) with modifications. Briefly, HEK293 cells were transfected with plasmid pcDNA3.1 (+) vectors (Invitrogen, Carlsbad, CA) containing SLC22A2 gene using lipofectamine 2000 reagent (Invitrogen). Colonies stably expressing hOCT2 were selected by G418 (500 μg/mL) and denoted as hOCT2-HEK293 cells, while cells transfected with empty pcDNA3.1 (+) vectors served as mock cells. All the cell lines were cultured in Dulbecco’s modified Eagle medium (DMEM, HyClone, Thermo Scientific, Waltham, MA) with 10% fetal bovine serum (HyClone, Thermo Scientific), penicillin (100 U/mL) and streptomycin (100 μg/mL) at 37°C with 5% CO2 and 95% humidity. For uptake study, cells were seeded on 24-well poly-D-lysine-coated plates (Corning Incorporated, Corning, NY) at a density of 1.5 x 10^5 cells/well and cultured for 3 days. 5 mM sodium butyrate was added 24 h before uptake experiments to induce protein expression of hOCT2.

The established hOCT2-HEK293 cell model was firstly validated by the uptake study of tetraethylammonium (TEA), a typical substrate of OCT2. The intracellular accumulation of TEA (20 μM) in hOCT2-HEK293 and mock cells were both determined for 2 min. Then, the uptake of guanfacine in hOCT2-HEK293 and mock cells were measured in a time-dependent experiment (guanfacine: 10 μM; uptake time: 30 s, 1, 2, 5, 10, 30 min), a concentration-dependent experiment (guanfacine: 1, 2, 5, 25, 50, 75, 100, 200 μM; uptake time: 2 min) and an inhibition study (guanfacine: 10 μM; cimetidine: 0.01, 0.1, 10, 100, 200, 1000, 2000 μM; uptake time: 2 min). In uptake studies, hOCT2-HEK293 cells and mock cells were placed in Hank’s balanced salt solution (HBSS: 143 mM NaCl, 4.69 mM KCl, 2.54 mM CaCl2, 1.18 mM KH2PO4, 1.17 mM MgSO4, 11.1 mM D-Glucose and 10 mM HEPES, pH 7.4) for 20 min. Then, the uptake was initiated by adding 0.3 mL of HBSS containing guanfacine in the absence or presence of cimetidine at 37°C. After incubation for designated times, uptake was terminated by aspirating the medium and adding ice-cold HBSS. The cells were washed with 1 mL ice-cold HBSS for three times. Finally, 200 μL of ultrapure water was added and the cells were lysed by three reduplicative freeze–thaw cycles. The intracellular concentration of TEA and guanfacine were determined by LC-MS/MS analysis. The uptake of TEA and guanfacine were normalized to the total protein contents in the cell lysates using a bicinchoninic acid assay (BCA Protein Assay Kit; Beyotime Institute of Biotechnology, Haimen, China).

In vivo pharmacokinetic study of guanfacine with cimetidine in rats

Male Sprague–Dawley rats weighed 180–220 g were purchased from Shanghai Sino-British Sippr/BK Experimental Animal Co. Ltd (Shanghai, China). Rats were given natural light and housed under controlled temperature and humidity. All rats were fasted overnight but with free access to water before administration. One day before the experiment, rats were put into separated metabolic cages for adaptation. The whole animal study was performed in accordance with the guidance of Animal Ethics Committee in China Pharmaceutical University (Nanjing, China).

Rats were randomly divided into four groups. Control group: guanfacine alone (1 mg/kg) + saline. Three experimental groups: (I) guanfacine (1 mg/kg) + cimetidine (25 mg/kg), (II) guanfacine (1 mg/kg) + cimetidine (50 mg/kg) and (III) guanfacine (1 mg/kg) + cimetidine (100 mg/kg). Three dosages of cimetidine or equivalent amount of saline were intravenously administered to rats. Ten minutes later, guanfacine (1 mg/kg) was administered to rats via the tail vein. Rats were kept in metabolic cages for spontaneously sampling of blood and urine. Blood samples were collected into heparinized tubes at 2, 5, 10, 30, 60, 90, 120, 180 and 240 min after intravenous injection of guanfacine. Plasma samples were obtained from the whole blood by centrifugation at 12,000 x g for 5 min at 4°C. Urine samples were collected over the time intervals of 0–2, 2–4, 4–6 and 6–12 h after dosing. All samples were stored at −20°C until LC-MS/MS analysis.
Data analysis

The pharmacokinetic parameters were calculated using Drug and Statistics software version 2.1.1 (Mathematical Pharmacology Professional Committee of China, Beijing, China) by non-compartmental analysis. The maximum plasma concentration ($C_{\text{max}}$) and time to $C_{\text{max}}$ ($t_{\text{max}}$) were directly observed by the obtained concentration–time data. The area under the curve from 0 to infinity ($AUC_{0-\infty}$) was calculated by the linear trapezoidal rule. The terminal elimination rate constant ($k_e$) was determined by linear regression with the terminal portion on the Ln (concentration)–time curve, and the eliminated half-life ($t_{1/2}$) was obtained by the equation $t_{1/2} = 0.693/k_e$. The apparent Michaelis–Menten constant ($K_m$) and the maximum uptake rate ($V_{\text{max}}$) of guanfacine uptake were obtained using the Michaelis–Menten equation as shown in Equation (1). The IC_{50} value of cimetidine on the uptake of guanfacine was calculated by nonlinear least square regression analysis with GraphPad Prism version 5 (GraphPad Software Inc., La Jolla, CA) according to Equation (2).

\[ V = \frac{V_{\text{max}}S}{K_m + S} \]  
\[ V = \frac{V_0}{1 + ([I]/IC_{50})^n} \]

where $V$ and $S$ are the uptake rates of guanfacine and guanfacine concentration, respectively.

where $V$ and $V_0$ are the uptake rates of guanfacine in the presence and absence of inhibitor, respectively, $[I]$ is the concentration of inhibitor and $n$ is the Hill coefficient.

The plasma clearance (CL_p) of guanfacine was calculated as follows:

\[ \text{CL}_p = \frac{\text{Dose}}{AUC_{0-\infty}} \]

The renal clearance (CL_r) of guanfacine was calculated by the following:

\[ \text{CL}_r = \frac{A_e(0-12\text{ h})}{AUC_{0-\infty}} \]

where $A_e(0-12\text{ h})$ is the total amount of guanfacine excreted into urine within 12 h.

All data are expressed as the mean ± SD. Data were analyzed statistically by an one-way analysis of variance (ANOVA) and Dunnett’s test. $p < 0.05$ was considered to be statistically significant.

Results

Uptake of TEA and guanfacine by hOCT2-HEK293 cells

To examine the reliability of the established hOCT2-HEK293 cell model, the uptake of TEA in hOCT2-HEK293 cells and mock cells was measured. As shown in Figure 1, the accumulation of TEA was much higher in transfected cells than that in mock cells.

To test the hypothesis that guanfacine is recognized by hOCT2, the transport characteristics of guanfacine in hOCT2-expressing HEK293 cells were investigated. The transport of guanfacine by hOCT2 was increased in a time-dependent manner (Figure 2). Guanfacine uptake in hOCT2-HEK293 cells was increased and gradually reached saturation with the increasing concentration of guanfacine (Figure 3). It is obvious that the cellular accumulation of guanfacine in hOCT2-HEK293 cells was significantly greater than that in vector-HEK293 cells. The $K_m$ value ($\mu$M) and $V_{\text{max}}$ value ($\text{nmol/mg protein/min}$) of guanfacine uptake by hOCT2-transfected cells were $96.19 ± 7.49$ and $13.03 ± 0.49$, respectively.

Inhibition of hOCT2-mediated guanfacine transport by cimetidine

To evaluate the inhibitory potency of cimetidine on guanfacine uptake mediated by hOCT2, the inhibition studies were performed by measuring the accumulation of guanfacine in hOCT2-HEK293 cells and mock cells in the presence of cimetidine, respectively. As shown in Figure 4, hOCT2-mediated uptake of guanfacine was significantly inhibited by cimetidine in a concentration-dependent manner. The IC_{50} value of cimetidine was calculated to be $93.82 ± 1.13 \mu$M.

Effects of cimetidine on the pharmacokinetics of guanfacine

To evaluate whether the in vitro interaction between guanfacine and cimetidine via OCT2 could also occur in vivo, we examined the effects of cimetidine on the
pharmacokinetics of guanfacine in rats. Three different dosages of cimetidine were co-administered with guanfacine to rats by intravenous injection to examine the concentration changes of guanfacine in rat plasma. As is shown in Figure 5, systemic exposure of guanfacine was markedly elevated by co-administration of cimetidine in a dose-dependent manner. The main pharmacokinetic parameters of guanfacine with or without cimetidine are summarized in Table 1. The pharmacokinetic characteristics of guanfacine were altered dose-dependently by cimetidine. Compared with the control group, AUC_{0-1} values of guanfacine in group I to III were increased markedly by an average of 54.5, 91.7 and 165.3% with a significant reduction of CL_{p} by an average of 31.1, 42.5 and 54.6%, respectively. The C_{max} of guanfacine was also increased obviously, while t\_1/2 of guanfacine was not significantly affected by cimetidine (Table 1).

To investigate whether the decreased plasma elimination and the elevated systemic exposure of guanfacine were caused by the inhibition of renal guanfacine excretion by cimetidine, the cumulative urinary excretion and renal clearance of guanfacine within 12 h after intravenous co-administration with cimetidine were compared with those in the control group. When guanfacine and cimetidine were spontaneously administered, the cumulative urinary excretion of guanfacine was significantly decreased (Figure 6). With increasing dosages of cimetidine, CL_{r} values of guanfacine in group I to III were markedly reduced by, on average, 77.4, 89.1 and 95.9%, respectively, in comparison with the control group (Table 1).

**Discussion**

Nowadays, drug combination has become increasingly essential for effective therapy. However, some drug combinations may result in unexpected or even harmful drug–drug interactions (DDIs), which will either reduce drug efficacy or enhance drug toxicity. In addition to metabolic enzymes, membrane transporters could also be expected as major determinants of the pharmacokinetics, response, and safety of drugs as is demonstrated by our previous reports (He et al., 2011, 2014; Lu et al., 2014; Zhao et al., 2012). Drug transporters expressed in the kidney are involved in the renal handling of numerous clinically used drugs, and inhibition of renal transporters will directly result in increased blood levels of these drugs, potentiating the risk of subsequent toxicity due to an impaired renal tubular secretion. Therefore, a comprehensive understanding of renal transporter-mediated DDIs in the elimination process is of great importance for the prevention of undesirable consequences.
Table 1. Mean pharmacokinetic parameters of guanfacine after intravenous injection at a dosage of 1 mg/kg in the absence or presence of cimetidine to rats (mean ± SD, n = 5).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control group</th>
<th>Experimental groups</th>
<th>I</th>
<th>II</th>
<th>III</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC_{(0–∞)} (μg/mL min)</td>
<td>12.1 ± 4.3</td>
<td></td>
<td>18.7 ± 0.6*</td>
<td>23.2 ± 1.8**</td>
<td>32.1 ± 2.5***</td>
</tr>
<tr>
<td>t_{1/2} (min)</td>
<td>69.94 ± 18.15</td>
<td></td>
<td>65.18 ± 6.43</td>
<td>68.71 ± 11.62</td>
<td>74.05 ± 2.90</td>
</tr>
<tr>
<td>C_{max} (μg/mL)</td>
<td>0.29 ± 0.10</td>
<td></td>
<td>0.40 ± 0.07*</td>
<td>0.41 ± 0.04*</td>
<td>0.56 ± 0.06***</td>
</tr>
<tr>
<td>CL_{p} (mL/kg/min)</td>
<td>82.91 ± 30.88</td>
<td></td>
<td>57.13 ± 1.90*</td>
<td>47.69 ± 2.62*</td>
<td>37.63 ± 7.35**</td>
</tr>
<tr>
<td>CL_{t} (mL/kg/min)</td>
<td>9.89 ± 4.96</td>
<td></td>
<td>2.24 ± 0.33**</td>
<td>1.08 ± 0.06**</td>
<td>0.41 ± 0.12***</td>
</tr>
</tbody>
</table>

*p < 0.05, **p < 0.01, ***p < 0.001 significant difference compared with control (guanfacine alone).

Figure 6. Urinary excretion curves of guanfacine after intravenous injection of 1 mg/kg guanfacine to rats in the absence or presence of cimetidine. Data are presented as mean ± SD (n = 5).

Guanfacine is an effective antihypertensive agent which has been found new applications in ADHD treatments recently. With negligible first-pass effect and minimal metabolism in human, guanfacine is mainly excreted as its intact form into urine through tubular secretion as well as glomerular filtration (Carchman et al., 1987; Cruz, 2010; Kiechel, 1980; Kirch et al., 1980; Sorkin & Heel, 1986). Therefore, DDIs mediated by renal transporters may play a critical role in guanfacine pharmacokinetics, thereby influencing the efficacy as well as the adverse effects of this drug. Since the physicochemical property of guanfacine is in line with the substrate characteristics of OCTs and OCT2 is the dominant isoform of OCTs in human kidney, it is likely that OCT2 may also play a certain role in the renal secretion of guanfacine, and consequently, DDIs via OCT2 would probably lead to an altered systemic exposure of guanfacine by affecting its renal clearance.

It is well known that cimetidine is a potent inhibitor for organic cation transport and has been widely used to evaluate the elimination-site DDIs involving OCTs (Abel et al., 2000; Bao et al., 2012; Chen et al., 2011; Feng et al., 2008; Shiga et al., 2000; Somogyi et al., 1992). Recent finding has demonstrated that the inhibition of tubular secretion of metformin by cimetidine was attributed mainly to OCT2 inhibition (Wang et al., 2008). Clinically, co-administration of cimetidine caused an increase by 24% in gabapentin AUC_{ss} with a decreased renal clearance as a result of OCT2 inhibition (Lal et al., 2010). It is also reported that cimetidine inhibited OCT2-mediated uptake of cisplatin, thus exhibiting protective effects against cisplatin-induced ototoxicity and nephrotoxicity (Ciarimboli et al., 2010; Katsuda et al., 2010). These evidences also indicate that OCT2-mediated DDIs appear to be clinically relevant.

In the present study, we explored the possible DDI of guanfacine via OCT2 by evaluating the effects of cimetidine on the in vitro uptake and in vivo pharmacokinetics of guanfacine. Cimetidine was selected in this study not only because it is a typical inhibitor for OCTs, but also because of its preferable affinity for both hOCT2 and rOct2 compared with that for other OCT subtypes in human and rats (Tahara et al., 2005). Firstly, the hOCT2-expressing HEK293 cell model was established and validated to be reliable for the subsequent uptake study of guanfacine based on the obtained results of TEA uptake study (Figure 1). Then, the in vitro transport of guanfacine was characterized using the established cell model and the inhibitory potency of cimetidine on guanfacine transport was also assessed. The results demonstrated that guanfacine uptake in hOCT2-HEK293 cells was considerably enhanced in both time- and concentration-dependent manner, in comparison with that in mock cells (Figures 2 and 3). The apparent affinity of guanfacine (K_{m} = 96.19 ± 7.49 μM) was much higher than that of model substrates for hOCT2 (metformin: K_{m} = 1.38 mM) (Kimura et al., 2005), suggesting that guanfacine is a high-affinity substrate for hOCT2. Besides, cimetidine exhibited a moderate inhibitory effect on hOCT2-mediated guanfacine uptake (Figure 4). Although the IC_{50} value of cimetidine for guanfacine transport (93.82 ± 1.13 μM) was much higher than the plasma concentration of guanfacine (6.3–23.8 μM) in individuals with normal kidney function (Larsson et al., 1982), the blood level of cimetidine was reported to be elevated due to a decreased renal clearance in elderly and patients with impaired renal function (Bjaeldager et al., 1980; Larsson et al., 1979; Redolfi et al., 1979). Therefore, the contribution of OCT2 in the interactions between guanfacine and cimetidine in these people should not be neglected. Furthermore, guanfacine extended-release formulation has been mainly applied for the treatment of ADHD in children and adolescents clinically. The maximum plasma concentration of guanfacine in children was reported to be 10.1 ± 7.1 ng/mL (0.04 ± 0.03 μM) after multiple 4-mg doses (Boellner et al., 2007), which is much lower than the concentration employed...
in the transport study (10 μM). According to a recent research (Thévenod et al., 2013), hOCT2 exhibited substrate-dependent inhibitor affinity. Therefore, a much lower IC50 value for inhibition by cimetidine might be observed if the concentration of guanfacine in human was adopted in the uptake study, which means that the inhibitory effects of cimetidine on guanfacine uptake could be stronger in this case.

In order to further evaluate the in vivo contribution of OCT2 in guanfacine elimination, we assessed the effects of cimetidine on guanfacine pharmacokinetics in rats. The results showed that the systemic exposure and renal clearance of guanfacine were both affected by cimetidine in a dose-dependent manner. When guanfacine and cimetidine were simultaneously administered intravenously to rats, the plasma concentration levels and AUC values of guanfacine were markedly increased with decreased CLp compared to the control group (Figure 5 and Table 1). Additionally, both the cumulative urinary excretion and CLu values of guanfacine in the presence of cimetidine were significantly lower than those in the absence of cimetidine (Figure 6 and Table 1). These results indicated that the elevated systemic exposure of guanfacine after pretreatment of cimetidine was attributed to the inhibition of the renal excretion of guanfacine by cimetidine, suggesting the role of rOcts in the renal secretion of guanfacine. It is reasonable to speculate that the interactions between guanfacine and cimetidine in rats was related to rOCT2 as well because of the much higher mRNA expression level of rOCT2 in rat kidney compared with that of rOCT1 and rOCT3 (Nakanishi et al., 2011). However, further studies using rOCT2-knockout rats or rOCT2-transfected cell models are needed to confirm this speculation. As mentioned above, urinary excretion of guanfacine is the major elimination pathway in human. Whereas, the accumulative urinary excretion of guanfacine in rats measured in this study was lower than that in human. This is probably due to the species difference between rats and human in the contribution of renal OCT2. Therefore, the role of OCT2 in the drug interactions between guanfacine and cimetidine as well as other cationic drugs warrants further investigations to understand the clinical significance of OCT2 in the renal disposition of guanfacine as well as to explore potential DDIs.

Conclusion

In the current study, we demonstrated for the first time that hOCT2 is involved in the basolateral uptake of guanfacine in human kidney. The in vivo interactions between guanfacine and cimetidine may also involve the inhibition of OCT2-mediated influx. Altogether, this study provided the first evidence that can be helpful to further understand the molecular mechanism underlying the renal tubular secretion of guanfacine.

Declaration of interest

The authors report no declaration of interest.

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References


