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Effects of Tetraalkylammonium Compounds with Different Affinities for Organic Cation Transporters on the Pharmacokinetics of Metformin

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ABSTRACT: The study sought to investigate the effects of tetraalkylammonium (TAA), inhibitors of the organic cation transporters (OCTs) with different affinities, on the pharmacokinetics of metformin. The inhibitory potentials of TAAs on the uptake of metformin were evaluated by determining IC_{50} values in MDCK cells over-expressing OCTs and, to assess *in vivo* drug interactions, metformin and TAAs were coadministered to rats. Uptake of metformin was facilitated by over-expression of hOCT1 and hOCT2 and showed saturable processes, indicating that metformin is a substrate of hOCT1 and hOCT2. The IC_{50} values of TAAs for hOCT2 were lower than hOCT1 and decreased with increasing alkyl chain length, indicating that the inhibitory potential of TAAs on metformin uptake was greater in hOCT2 than in hOCT1 and increased with increasing alkyl chain length. The plasma concentration of metformin was elevated by the coadministration of tetrapropylammonium (TPrA) and tetrapentylammonium (TPeA), but not by tetramethylammonium (TMA) or tetraethylammonium (TEA). However, the plasma concentrations of TMA, TEA and TPrA were not changed by the coadministration of metformin. In conclusion, *in vivo* drug interactions between metformin and TAAs were caused only when metformin was coadministered with TAAs showing higher affinities for OCTs. Copyright © 2007 John Wiley & Sons, Ltd.

Key words: drug interaction; metformin; tetraalkylammonium (TAA); organic cation transporter (OCT); affinity

Introduction

Metformin is effective in the primary therapy of type 2 diabetes mellitus with obesity or hyperlipidemia and can also be used as an add-on therapy in patients with diabetes uncontrolled by sulfonylureas and diet [1–3]. Metformin is extensively eliminated in the kidney via tubular secretion mediated by organic cation transporters (OCTs); approximately 79–86% of an intravenous dose is recovered in urine unchanged in humans [4,5]. Thus, OCTs play a key role in the pharmacokinetics of metformin.

Metformin is a better substrate for the renal OCT2 than the hepatic OCT1 [6,7]. Consequently, OCT2 is the most likely candidate for controlling renal excretion of metformin [7,8]. OCT1 is known to be responsible for hepatic uptake of metformin and lactic acidosis [9,10], a severe adverse effect of metformin characterized by low arterial pH (<7.35) and elevated arterial lactate levels (5.0 mEq/l in humans) [11,12].

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Lactic acidosis may be caused by the overproduction of lactate through inhibition of mitochondrial respiration and increased anaerobic glycolysis [1]. Lactic acidosis is also associated with renal dysfunction and because renal secretion is the major elimination route of metformin, renal dysfunction will likely cause a major increase in the plasma concentration of metformin, resulting in more lactic acidosis [13]. Thus, increasing the plasma concentration of metformin by inhibiting OCTs may reduce hepatic uptake and increase the incidence of lactic acidosis. Consequently, interactions between metformin and substrates for OCTs should be carefully considered. As more than 30% of clinically used drugs are organic cations, drug interactions between metformin and substrates of OCTs are highly plausible [14].

Cimetidine, which is a substrate of OCT1 and OCT2 [15], significantly increased the *AUC* of metformin and reduced its renal clearance [10,16]. The total urinary recovery of metformin was not changed by the coadministration of cimetidine, suggesting that competitive inhibition via renal tubular secretion of metformin by cimetidine can explain the drug interaction between metformin and cimetidine. However, metformin had no effect on the disposition of cimetidine [16]. These results suggest that drug interactions between substrates for OCTs can be different, depending on the properties of substrates, such as affinity for the transporter.

A series of tetraalkylammonium (TAA) compounds has been reported, all of which are organic cations that show increasing lipophilicity with increasing alkyl chain length [17]. TAA compounds also showed a good correlation between inhibition potency (e.g. IC_{50} value) in inhibiting tetraethylammonium (TEA) uptake in HeLa cells expressing hOCT1 and alkyl chain length [18]. The tendency for a correlation between the inhibitory potency of TAA compounds and alkyl chain length was quite similar in OCT1 from different species, such as mice, rats, rabbits, and humans, even though the apparent K_i value was different among species [19]. However, this tendency was not seen in hOCT2-overexpressing oocytes, indicating that the substrate specificity is different in the isoforms of OCT [19]. For example, the K_m values for TEA in an hOCT1 overexpressing system had a 229 μM range, those in an hOCT2 over-expressing system were 31–431 μM. Likewise, the K_m values of MPP^+ in an hOCT1 over-expressing system were 14.6–32 μM, those in an hOCT2 over-expressing system were 1.2–19 μM, and those in an hOCT3 over-expressing system were 47 μM range (this information is available at TP–search data base, http://133.9.194.61/tp-search/).

Thus, the study sought to characterize *in vitro* inhibitory potency of TAA compounds on the uptake of metformin in the three isoforms of OCT and to evaluate *in vivo* drug–drug interactions between metformin and TAA compounds with different affinity to OCTs. This study will help in understanding the transporter-based drug interactions between substrates and/or inhibitors of transporters.

Materials and Methods

Chemicals

[^{14}C]Metformin was purchased from Moraveck Biochemicals Inc. (Brea, CA) and unlabeled metformin (1,1-dimethylbiguanide hydrochloride) and antipyrin were purchased from Sigma–Aldrich Chemical Co. (St Louis, MO). Tetramethylammonium bromide (TMA), tetraethylammonium chloride (TEA), tetrabutylammonium tribromide (TBA), tetrapentylammonium bromide (TPeA) and tetraheptylammonium bromide (THeA) were purchased from Sigma–Aldrich Chemical Co. and tetrapropylammonium bromide (TPrA) and tetrahexylammonium bromide (THA) were obtained from Lancaster Synthesis (Morecambe, UK). All other chemicals were reagent grade and all solvents were HPLC grade.

Animals

The experimental protocols involving animals were approved by the Animal Care and Use Committee of the College of Pharmacy, Seoul National University, consistent with the US National Institutes of Health guidelines.

Male Sprague–Dawley rats (weighing 260–295 g) were purchased from Dae–Han Biolink

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(Daejeon, Korea). All rats were provided with food (Sam Yang Co., Seoul, Korea) and water *ad libitum* and were maintained in a light-controlled room (light: 07:00–19:00, dark: 19:00–07:00), kept at a temperature of $22 \pm 2^{\circ}C$ and a relative humidity of $55 \pm 5\%$ (Animal Center for Pharmaceutical Research, College of Pharmacy, Seoul National University, Seoul, Korea).

Inhibitory effect of TAA on the uptake of metformin

For the construction of stable hOCT1-, hOCT2- and hOCT3-overexpressing cells, the MDCK cells were transfected with pcDNA3.1 plasmids that carried the genes for hOCT1, hOCT2 and hOCT3, respectively, using Lipofectamine 2000. The stable cells were selected for resistance to 800 μg/ml geneticin for 3 weeks. The expression levels of hOCT1, hOCT2 and hOCT3 were verified by Western blot analysis using respective polyclonal antibodies (Santa Cruz Biotechnology, Santa Cruz, CA).

MDCK cells and hOCT1-, hOCT2-, and hOCT3-overexpressing MDCK cells (MDCK-hOCT1, -hOCT2, -hOCT3) were maintained at 37°C in a humidified atmosphere of 5% CO_2/95% air and grown in Dulbecco's modified Eagle's medium supplemented with 10% (v/v) fetal bovine serum, L-glutamine (2 mM) and penicillin–streptomycin (100 U/ml). For experiments, 10^4 cells were seeded in 96-well plates. After the cells reached 95% confluence, the growth media were discarded and the attached cells were washed with Eagle's media and preincubated for 1 h in serum-free DMEM at 37°C. The uptake of radiolabeled drug was initiated by the addition of medium (200 μl) containing 1 μM [^{14}C]metformin, which was added to the cells in the presence or absence of various concentrations of TAA compounds at 37°C. The cells were washed three times with 200 μl of ice-cold phosphate-buffered saline immediately after placing the plates on ice. The radioactivity of the [^{14}C]metformin in the cells was measured by liquid scintillation counting after lysing the cells with 50 μl of cell culture lysis reagent [100 mM KH_2PO_4 (pH 7.8), 1 mM EDTA, 7 mM 2-mercaptoethanol, 1% (v/v) Triton X-100, 10% (v/v) glycerol; Promega, Madison, WI].

Pharmacokinetic studies of metformin and TAA

Male Sprague–Dawley rats were divided into five groups, four rats per group. Groups 1–4 were given 30 μmol/ml/kg of metformin with 30 μmol/ml/kg of TMA, TEA, TPrA or TPeA, respectively. Group 5 was given 30 μmol/ml/kg of metformin alone. Under anesthesia with ketamine (50 mg/kg, i.p. injection), the femoral arteries and veins of rats were cannulated with PE-50 polyethylene tubing (Intramedic, Sparks, MD). After recovery from surgery, metformin alone, or coadministration of metformin and TAA compound were injected via the femoral vein of rats. An aliquot of blood (250 μl) was collected via the femoral artery at 0, 1, 3, 5, 10, 30 and 60 min after the intravenous administration of metformin alone, or metformin and TAA compound solution. An aliquot (150 μl) of heparinized normal saline (20 units/ml) was used to flush each cannula immediately after each blood sample to prevent blood clotting, and fluid loss was compensated by an injection of normal saline at each collection via the femoral vein. Body temperature was maintained with a heat lamp. Blood samples were centrifuged immediately and an aliquot (100 μl) of each plasma sample was stored at −70°C until analysed.

Determination of plasma concentration of metformin

The plasma concentrations of metformin were determined by an HPLC method based on the report of Zarghi *et al.* [20]. An aliquot of plasma (100 μl) was added to an aliquot (20 μl) of acetonitrile containing 100 μg/ml of antipyrin (an internal standard) and a 200 μl acetonitrile. After vortex-mixing for 5 min, the supernatant was transferred to a clean tube and evaporated under a gentle stream of nitrogen gas. The residue was reconstituted in a 170 μl aliquot of distilled water, and a 100 μl aliquot was injected directly into the HPLC system. The chromatographic system consisted of a pump (Waters 515; Waters Corporation, Milford, MA), an automatic injector (Waters 717 plus autosampler) and a UV detector (Waters 2487). A C18 column (μBonapak, 3.9×150 mm, 10 μm; Waters Corporation) was eluted with a mobile phase consisting of 10 mM

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phosphate buffer (pH 7.0) containing 10 mM sodium dodecyl sulfate:acetonitrile (60:40 v/v) at a flow rate of 1.0 ml/min. Metformin was monitored at 237 nm. The retention times of metformin and antipyrin were approximately 4.3 and 2.5 min, respectively. The calibration curve from the standard samples was linear over the concentration range 3–360 μM and the quantification limit of metformin in rat plasma was 3 μM. The interday coefficients of variation were below 11.48%.

Determination of plasma concentration of TAAs

The concentrations of TMA, TEA and TPrA in plasma were quantified by LC-MS system. A 20 μl aliquot of plasma was deproteinized with 100 μl of acetonitrile. A 5 μl aliquot of reconstituted residue of the supernatant was injected directly into the LC-MS system. The HPLC system consisted of a Waters 2695 separation module with quaternary pump and autoinjector. A Gemini C18 analytical column (2.0 × 150 mm, 3 μm; Phenomenex, Torrance, CA) was used. The flow rate of the mobile phase (i.e. MeOH:DDW = 7:3 v/v) was set at 0.2 ml/min. A Finnigan LCQ ion-trap mass spectrometer equipped with an ESI source was used for mass analysis and detection. Mass spectrometric analysis was performed in the positive ion mode and set up in the selected ion monitoring (SIM) mode. On the basis of the full-scan mass spectra of each analyte, the most abundant ion was selected and the mass spectrometer was set to monitor the ions as follows: *m/z* 74.0 for TMA, *m/z* 130.3 for TEA and *m/z* 186.2 for TPrA. For all compounds, calibration studies indicated that the detector response was linear over 2–150 μM for TMA and TEA, 0.2–200 μM for TPrA below 15.0% of interday coefficients of variation.

Data analysis and statistics

Non-compartmental pharmacokinetic analysis was performed using the WinNonlin software (version 5.1; Pharsight Co., Mountain View, CA). The area under the plasma concentration-time curve (*AUC*) was calculated using the linear trapezoidal method. The area from the last datum point to time infinity was estimated by dividing the last measured concentration in plasma by the terminal rate constant. The terminal elimination half-life ($t_{1/2}$), the systemic clearance (*Cl*) and volume of distribution (V_d) were obtained. The initial plasma concentration (C_o) is the estimated plasma concentration of metformin at time 0. A value of $p < 0.05$ was deemed to be statistically significant, using a Mann Whitney test between the two means for unpaired data. All data are expressed as the mean ± SD.

Results

Concentration dependency of metformin in OCT-expressing MDCK cells

The concentration dependency of metformin uptake was determined to characterize metformin uptake in MDCK cell lines stably expressing the hOCT1, hOCT2 and hOCT3 proteins, which was confirmed by Western blot analysis (Figure 1C). Metformin uptake mediated by hOCT1 and hOCT2 was saturable, while that via hOCT3 was not (Figure 1A). The values of K_m and V_{max} obtained from the Michaelis–Menten equation [i.e. $v = V_{max} \times C/(K_m + C)$] were 1060 ± 263 μM and 440.8 ± 118 pmol/min/mg protein, respectively, for hOCT1, and 1072 ± 305 μM and 1530 ± 199 pmol/min/mg protein, respectively, for hOCT2. The Eadie–Hofstee transformation also revealed saturable processes in the uptake of metformin via hOCT1 and hOCT2, but not in hOCT3 cells (Figure 1B).

Inhibitory effect of TAA compounds on the uptake of metformin via OCTs

To determine the IC_{50} values of TAA compounds, the inhibitory effect of TAA compounds on the uptake of metformin in MDCK cells overexpressing the hOCT1, hOCT2 and hOCT3 proteins was examined; a representative inhibition profile of TPrA is shown in Figure 2A. Data were fitted to an inhibitory effect E_{max} model [i.e. $v = E_{max} - E_{max} \times C/(IC_{50} + C)$] and the resulting IC_{50} values are shown in Table 1. The IC_{50} values for hOCT2 were lower than those for hOCT1 when the same compound was assessed except for TPeA. Moreover, the IC_{50} values for both

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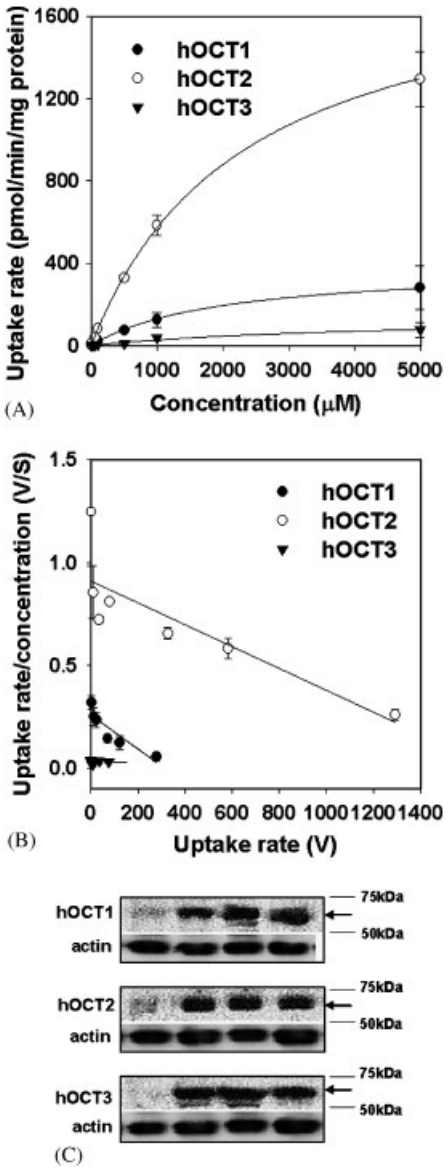

Figure 1. Concentration dependency of metformin uptake in hOCT1-, hOCT2- and hOCT3-overexpressing MDCK cell line. (A) Uptake of [^{14}C]metformin was measured for 10 min with the concentration range of 10–5000 μM. The hOCT1-, hOCT2- and hOCT3- mediated uptake of metformin was calculated by subtracting the metformin uptake in vector transfected cells from that in hOCT1-, hOCT2- and hOCT3-overexpressing MDCK cells. Each point represents the mean ± SD of four independent experiments. (B) The Eadie-Hofstee transformation of the initial uptake rate. (C) Western blot analysis of MDCK cells that stably express hOCT1, hOCT2 and hOCT3. Lane 1 represents vector transfected control cells and lanes 2–4 represent three different clones that express hOCT1, hOCT2 and hOCT3, respectively

hOCT1 and hOCT2 decreased as the alkyl chain length increased and showed a good correlation with alkyl chain length in a semilogarithmic plot [i.e. $\log(IC_{50}) = -0.6953x + 4.056$, $r^2 = 0.9456$, $p < 0.001$ for hOCT1; $\log(IC_{50}) = -0.5905x + 3.3489$, $r^2 = 0.9531$, $p < 0.001$ for hOCT2] (Figure 2B).

To evaluate the inhibitory effect of TAA compounds on the passive uptake of metformin, the metformin uptake was measured in the presence of TAA compounds in vector transfected MDCK cells which did not express hOCTs. The presence of TAA compounds did not significantly inhibit the passive uptake of metformin (Figure 2C), suggesting that TAA compounds seem to modulate metformin uptake by inhibiting hOCT1 and hOCT2 transporters directly rather than influence membrane fluidity.

Drug interactions between TAA and metformin in rats

To assess the consequences of different IC_{50} values of TAA compounds in *in vivo* drug interactions between metformin and TAA compounds, metformin and TAA compounds with different IC_{50} values were coadministered intravenously into rats. Plasma concentrations of metformin were elevated by the presence of TPrA and TPeA, but not by TMA or TEA, compared with the control group (Figure 3). The pharmacokinetic parameters also confirmed that *in vivo* drug interactions occurred when TPrA and TPeA were coadministered with metformin (Table 2). In other words, the values of C_o and *AUC* for metformin in the presence of TPrA and TPeA increased 1.78- and 1.79-fold, respectively, for TPrA and increased 1.87-fold for TPeA. The values of *Cl*, $t_{1/2}$ and V_d for TPrA and TPeA decreased 1.79-, 1.70- and 2.98-fold, respectively, for TPrA and decreased 1.81-, 1.56- and 2.84-fold, respectively, for TPeA. However, coadministered TMA and TEA did not change the pharmacokinetic parameters of metformin.

To investigate whether the plasma concentrations of TAA compounds with different affinities to OCTs were different and whether the pharmacokinetic behaviors of TAAs were changed by the coadministration of metformin, the study also determined the plasma concentration of TMA,

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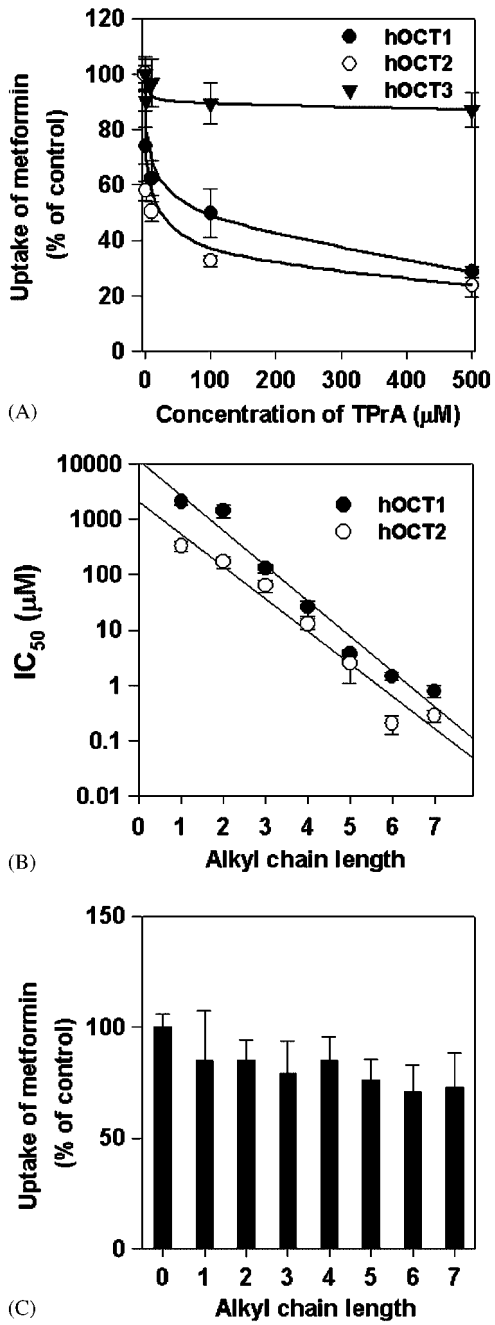
TEA, and TPrA, which showed the most dramatic decrease in IC_{50} value, in the absence or the presence of metformin. The plasma concentration profiles were similar among TMA, TEA and TPrA and the pharmacokinetic parameters such as *AUC* and *Cl* were not different among TMA, TEA and TPrA. Moreover, the plasma concentrations of TAAs were not changed by the coadministration of metformin (Figure 4).



Discussion

As the general consensus holds that transporters play key roles in drug absorption, disposition, toxicity and efficacy, transporter-based drug interaction may lead to pharmacokinetic changes in substrates coadministered and increased toxicity or lack of efficacy [21]. For example, metformin undergoes extensive renal excretion via OCT2, without significant metabolism [7,8]. The glucose-lowering effect of metformin and lactic acidosis, an adverse effect of metformin, are associated with OCT1 [9,10]. In addition, drug interactions of metformin through OCT1 and OCT2 may alter the pharmacokinetics and efficacy or toxicity of metformin. Therefore, methods to predict the potential for significant interactions between substrates for OCTs that act as inhibitors need to be explored. A goal of this study was to evaluate *in vivo* drug interactions between two substrates in terms of the affinity to their respective transporters.

Metformin is a substrate for hOCT1 and hOCT2 but not for hOCT3, which is consistent with the previous report of Kimura *et al*. [6]. Kinetic parameters demonstrated that metformin showed similar affinity to hOCT1 and hOCT2 with a different V_{max} value, which resulted in

Figure 2. (A) Concentration dependent inhibition of [^{14}C]metformin uptake by TPrA in hOCT1-, hOCT2- and hOCT3-overexpressing MDCK cell line. Initial uptake rates were determined in the presence of various concentrations of TPrA. Each point represents the mean ± SD of four independent experiments. (B) Relationship between IC_{50} values in inhibiting [^{14}C]metformin uptake in hOCT1- and hOCT2-overexpressing MDCK cells and alkyl chain length of TAA compounds. The IC_{50} values showed a good correlation with alkyl chain length in a semilogarithmic plot [i.e. $\log(IC_{50}) = -0.6593x + 4.056$, $r^2 = 0.9456$, $p < 0.001$ for hOCT1; $\log(IC_{50}) = -0.5905x + 3.3489$, $r^2 = 0.9531$, $p < 0.001$ for hOCT2, respectively]. (C) Inhibitory effect of TAA compounds on metformin uptake in vector transfected MDCK cells. Initial uptake rates of metformin were measured in the presence of 100 μM of TAA compounds with various alkyl chain lengths. Each data point represents the mean ± SD of four independent experiments

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Table 1. Inhibitory effect of TAA on the uptake of metformin in hOCT1- and hOCT2-overexpressing MDCK cell line

TAA	Alkyl chain length	IC_{50} (μM)	
		hOCT1	hOCT2
TMA	1	2071 ± 231	327.7 ± 70.6[a]
TEA	2	1405 ± 374	167.9 ± 40.3[a]
TPrA	3	129.52 ± 23.2	62.53 ± 14.1[a]
TBA	4	25.60 ± 7.7	12.99 ± 2.9[a]
TPeA	5	3.63 ± 0.7	2.46 ± 1.4
THA	6	1.47 ± 0.2	0.21 ± 0.07[a]
THeA	7	0.78 ± 0.2	0.28 ± 0.07[a]

TMA, tetramethylammonium; TEA, tetraethylammonium; TPrA, tetrapropylammonium; TBA, tetrabutylammonium; TPeA, tetrapentylammonium; THA, tetrahexylammonium; THeA, tetraheptylammonium.

[a] $p < 0.05$, Significantly different from MDCK-hOCT1.

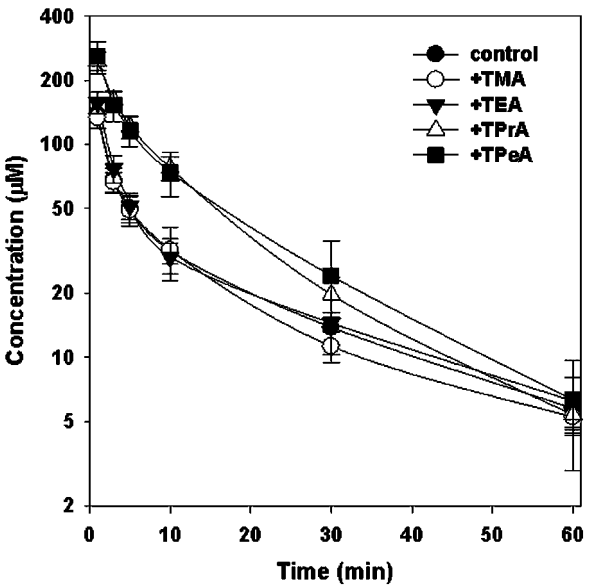

Figure 3. Plasma concentration profile of metformin in rats following an i.v. bolus administration of 30 μmol/ml/kg of metformin alone (●) and coadministration of 30 μmol/ml/kg metformin with 30 μmol/ml/kg of TMA (○), TEA (▼), TPrA (△), and TPeA (■), respectively. Each data point represents the mean ± SD of four rats

3.7-fold higher intrinsic clearance via hOCT2 than hOCT1. Considering kidney specific expression of hOCT2 and renal excretion is a major elimination route for metformin, the inhibition of hOCT2 rather than hOCT1 mediating metformin transport may cause changes in pharmacokinetics of metformin. For example, the plasma concentration profile of metformin in $Oct1^{-/-}$ mice was not significantly different from that in control [22] while the plasma concentration profile of TEA was significantly increased in $Oct1/2^{-/-}$ mice, which is a better reflection of hOCT2 [23].

A series of TAA compounds with a common quaternary ammonium structure but different affinity, depending on the alkyl chain lengths, to both the hOCT1 and hOCT2 transporters, suggest that TAA compounds are good model inhibitors for OCTs when the inhibitory effect of such compounds on the pharmacokinetics of metformin was measured *in vivo*. The results indicated that the IC_{50} values of TAA compounds for the uptake of metformin mediated by both hOCT1 and hOCT2 were decreased gradually with increasing alkyl chain length (Figure 2B). However, Dresser *et al.* [19] reported that the IC_{50} values of TAA compounds for hOCT2 mediating MPP^+ uptake were similar regardless of alkyl chain length. This discrepancy may be explained by the use of different probe drugs (e.g. MPP^+ versus metformin) because the K_m values of MPP^+ in hOCT1- and hOCT2-expressing HEK293 cells were 32 μM and 7.8 μM, respectively [15] and the K_m values for metformin in hOCT1- and hOCT2-expressing MDCK cells were 1060 ± 263 μM and 1072 ± 305 μM, respectively (in this study). These results suggested that the IC_{50} value might be influenced by the relative affinities between the probe substrate and inhibitors for hOCT1 and hOCT2. Likewise, the IC_{50} value might be also influenced by the inhibitor specificity to hOCT1 and hOCT2. For example, the IC_{50} value of MPP^+ for hOCT1 was greater than that for hOCT2 (12.3 μM and 2.4 μM, respectively) [24] and the IC_{50} values of metformin on cimetidine uptake were not different between hOCT1 and hOCT2, while the inhibitory potential of phenformin, a higher affinity substrate for hOCT1 compared with metformin, was greater in hOCT1 than in hOCT2 when measured with cimetidine as a substrate [19]. Although the underlying mechanism is not fully elucidated, a different specificity of substrates or inhibitors to hOCT1 and hOCT2 may explain the reason that the IC_{50} values of TAA compounds for hOCT1 are greater than those for hOCT2 except for TPeA in our study and the IC_{50} value of TMA for

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Table 2. Pharmacokinetic parameters of metformin following an i.v. bolus administration of metformin (30 μmol/ml/kg) to rats in the presence and absence of TAA (30 μmol/ml/kg)

	Control	+TMA	+TEA	+TPrA	+TPeA
C_o (μM)	138.1 ± 20.0	133.3 ± 3.8	157.3 ± 18.1	245.8 ± 24.3[c]	258.1 ± 43.6[b]
AUC (μM*min)	1615.4 ± 205.1	1497.7 ± 159.6	1710.3 ± 307.0	2886.4 ± 387.6[b]	3018.9 ± 735.5[a]
Cl (ml/min/kg)	19.0 ± 2.3	20.4 ± 2.1	18.3 ± 4.0	10.6 ± 1.5[c]	10.5 ± 2.6[c]
$t_{1/2}$ (min)	20.7 ± 2.2	18.5 ± 2.3	22.2 ± 2.5	12.2 ± 0.6[c]	13.3 ± 2.5[c]
V_d (ml/kg)	425.9 ± 73.9	420.2 ± 95.9	410.6 ± 15.7	142.9 ± 14.3[c]	150.0 ± 18.8[c]

[a] $p < 0.05$, Significantly different from control group.
[b] $p < 0.01$, Significantly different from control group.
[c] $p < 0.001$, Significantly different from control group.

hOCT1 was higher than that for hOCT2, whereas the inhibitory potential of TBA was greater in hOCT1 than hOCT2 [19].

According to Dresser *et al.* [25], the tendency for a correlation between the inhibitory potency of TAA compounds and alkyl chain length was quite similar in OCT1 transporters from different species, such as mice, rats, rabbits and humans, even though the apparent K_i value was different among them when measured with MPP^+ as a substrate for OCT1. However, the metformin uptake mediated by OCT2 showed similar characteristics between human and rat compared with OCT1 (e.g. the K_m values for hOCT1 and rOCT1: 1.47 ± 0.19 mM and 3.73 ± 0.15 mM, respectively; the K_m values for hOCT2 and rOCT2: 0.99 ± 0.03 mM and 0.63 ± 0.09 mM, respectively) [6]. Thus, although the experimental systems and species were not perfectly matched, after determining the IC_{50} values of TAA compounds on metformin uptake via hOCT1- and hOCT2-overexpressing cell lines, the study continued to evaluate *in vivo* drug interactions between metformin and TAA compounds with different IC_{50} values. As a result of *in vivo* pharmacokinetic experiments, the plasma concentration of metformin significantly increased after coadministration of TPrA and TPeA, but not of TMA or TEA. Since metformin and TAA are not protein bound in either plasma or liver cytosol and not metabolized [4,5,17,26], the changes in pharmacokinetics of metformin seems to reflect the interactions in distribution and elimination process of metformin with TAA in relation to OCT1 and OCT2. Therefore, the increased plasma concentration of metformin caused by TPrA, but not by TMA or TEA, may be attributed to the differential inhibitory potential of TAA compounds to both OCT1 and OCT2 because the plasma concentration profiles of TMA, TEA and TPrA were comparable (i.e. 124 ± 16 μM, 137 ± 25 μM and 146 ± 15 μM, respectively, at 1 min after the administration). The plasma concentration of TPrA at 5 min after the administration was higher than IC_{50} value for hOCT2, whereas the plasma concentrations of TMA and TEA were lower than IC_{50} values even at the earliest time point. These results, taken together, suggest that alterations in the pharmacokinetics of metformin may occur when an inhibitor with a lower IC_{50} value is coadministered and exists at a higher concentration. As metformin is primarily excreted into the urine, OCT2 is a major transporter expressed in the kidney, and the Cl_{int} of OCT2 is much higher than that of OCT1 [6], selection of an inhibitor with a low IC_{50} value for hOCT2 seem to be important to examine possible drug interactions in the renal elimination of metformin.

The values of C_o and *AUC* for metformin significantly increased and those of *Cl* significantly decreased in the presence of TPrA and TPeA. As metformin was recovered almost entirely in an unchanged form in urine, decreased *Cl* may reflect decreased renal excretion through competitive inhibition of OCTs. The value of V_d also decreased, which may have been attributable to a decreased tissue distribution of metformin to the liver, intestine and kidney via inhibition of OCTs, which facilitated the uptake process and tissue distribution. However, unexpectedly, the value of the terminal

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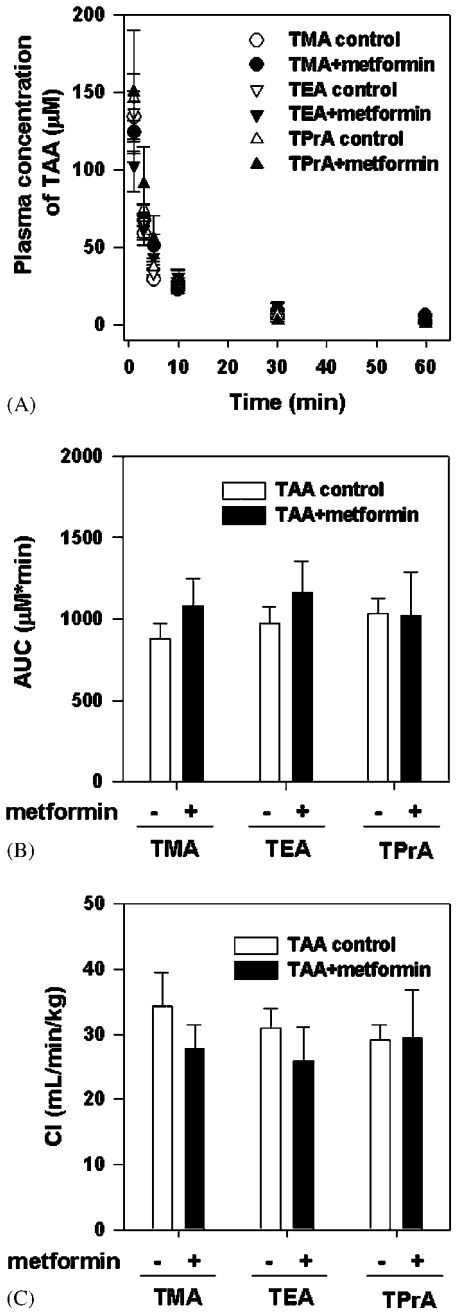

Figure 4. (A) Plasma concentration profile of TAA in rats following an i.v. bolus administration of 30 μmol/ml/kg of TMA (○), TEA (▽), TPrA (△) alone and coadministration of 30 μmol/ml/kg metformin and TAA (● for TMA,▼ for TEA, ▲ for TPrA). Each point represents the mean ± SD of three rats. (B and C) The values of *AUC* (B) and *Cl* (C) of TAA in rats after i.v. bolus injection of TMA, TEA and TPrA in the absence (open bar) and the presence (closed bar) of metformin. *AUC* and *Cl* were calculated from the data in Figure 4A

elimination half-life ($t_{1/2}$) of metformin significantly decreased in the presence of TPrA and TPeA compared with those of the control. As the drug interaction between metformin and TPrA or TPeA was mediated by OCT1 and OCT2, which are located in the basolateral membrane of the liver and kidney, the plasma concentration of TPrA or TPeA was very important for the competitive inhibition of metformin disposition. According to previous reports, quaternary ammonium compounds are eliminated very quickly from plasma and accumulate in the liver and kidney after intravenous injection [17,27]. Our results also showed that the plasma concentration of TPrA and TPeA was maintained higher than their IC_{50} value only for 5 min and 30 min (i.e. 74.5 ± 3.7 μM and 2.57 ± 0.68 μM, respectively). Thus, these OCT-based drug interactions may be maintained only for a short time, and as a result, the disposition of metformin was delayed for a short time and underwent its usual elimination process, which may have been due to the apparently decreased $t_{1/2}$ of metformin through coadministration with TPrA or TPeA. Contrary to the case of metformin, the pharmacokinetics of TPrA as well as TMA and TEA were not changed by the concomitant administration of metformin.

In conclusion, along with screening an inhibitory effect of a series of TAA compounds on the *in vitro* uptake of metformin, the *in vitro*/*in vivo* correlation for organic cation transporters appears to be reasonable for OCT-based interactions between metformin and higher affinity inhibitors, TAA compounds. However, interpretation should be applied with care because the transporter based drug interaction can occur when higher affinity inhibitors are present at appropriate concentrations.

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