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The Effect of Nizatidine, a MATE2K Selective Inhibitor, on the Pharmacokinetics and Pharmacodynamics of Metformin in Healthy Volunteers

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Abstract

Background and Objectives—In the proximal tubule, basic drugs are transported from the renal cells to the tubule lumen through the concerted action of the H^+/organic cation antiporters, multidrug and toxin extrusion 1 (MATE1) and 2K (MATE2K). Dual inhibitors of the MATE transporters have been shown to have a clinically relevant effect on the pharmacokinetics of concomitantly administered basic drugs. However, the clinical impact of selective renal organic cation transport inhibition on the pharmacokinetics and pharmacodynamics of basic drugs, such as metformin, is unknown. This study sought to identify a selective MATE2K inhibitor *in vitro* and to determine its clinical impact on the pharmacokinetics and pharmacodynamics of metformin in healthy subjects.

Methods—A strategic cell-based screen of 71 U.S. Food and Drug Administration (FDA)-approved medications was conducted to identify selective inhibitors of renal organic cation transporters that are capable of inhibiting at clinically relevant concentrations. From this screen, nizatidine was identified and predicted to be a clinically potent and selective inhibitor of MATE2K-mediated transport. The effect of nizatidine on the pharmacokinetics and pharmacodynamics of metformin was evaluated in 12 healthy volunteers in an open-label, randomized, two-phase crossover drug-drug interaction (DDI) study.

Results—In healthy volunteers, the MATE2K-selective inhibitor, nizatidine, significantly increased the apparent volume of distribution, half-life and hypoglycemic activity of metformin. However, despite achieving unbound maximum concentrations greater than the *in vitro* inhibition potency (IC_{50}) of MATE2K-mediated transport, nizatidine did not affect the renal clearance or net secretory clearance of metformin.

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Conflicts of Interest The authors have no conflicts of interest that might be relevant to the content of this manuscript

7 Compliance with Ethical Standards

Ethical Approval The Committee on Human Research at UCSF (Institutional Review Board [IRB] 11-06968) approved the clinical DDI study, and all subjects were recruited directly from the Study of Pharmacogenetics in Ethnically Diverse Populations (IRB 10-03167). Informed consent was obtained from all study participants.

Conclusion—This study demonstrates that a selective inhibition of MATE2K by nizatidine, affected the apparent volume of distribution, tissue levels and peripheral effects of metformin. However, nizatidine did not alter systemic concentrations or the renal clearance of metformin, suggesting that specific MATE2K inhibition may not be sufficient to cause renal DDIs with basic drugs.

1 Introduction

In the proximal tubule of the kidney, basic drugs are transported from the blood to the lumen of the kidney by organic cation transporter 2 (OCT2) and are eliminated to the urine by the concerted action of the H+/organic cation antiporters, multidrug and toxin extrusion 1 (MATE1) and 2K (MATE2K). Broadly selective inhibitors of multiple organic cation transporters (e.g., cimetidine for OCT2/MATE1/MATE2K, pyrimethamine for MATE1/MATE2K) have been shown to have a clinical impact on the pharmacokinetics of concomitantly administered organic cations (e.g., metformin, procainamide, ranitidine) through reduction in their renal clearance [1–4]. However, the clinical impact of selective inhibition of a single organic cation transporter on the pharmacokinetics and pharmacodynamics of basic drugs is unknown.

MATE2K is believed to be an important renal transporter for many drugs. In comparison to MATE1, which is expressed in multiple tissues (e.g., kidney, liver, muscle), MATE2K is predominately expressed in the kidney [5], and at equivalent or higher levels than MATE1 (S.W. Yee, A. Chhibber, D.L. Kroetz and K.M. Giacomini, unpublished data). MATE2K also specifically transports some drugs (e.g., oxaliplatin), which do not appear to be substrates of MATE1 [6, 7]. Studies from our laboratory have shown that a common MATE2K promoter variant (g.-130G>A, rs12943590) is associated with poor response to the biguanide, metformin in type 2 diabetic subjects [8, 9]. Taken together, these data suggest that MATE2K is important for the renal elimination of many basic drugs including metformin.

As transporter-mediated drug-drug interactions (DDIs) occur in clinical situations and have an impact on pharmacokinetics and drug safety, regulatory agencies in the United States (U.S.) and European Union have issued guidances that recommend using *in vitro* transporter studies to inform the decision of when to conduct a clinical DDI study. The U.S. Food and Drug Administration (FDA) recommends that a clinical investigation of a transporter-mediated drug interaction should be conducted when the I_{fu}/IC_{50} ratio (maximum plasma concentration [C_{max}] of the inhibitor that is not bound to plasma proteins [$C_{max,u}$] divided by the concentration associated with half the maximum inhibition in an *in vitro* assay) of the new molecular entity is ≥ 0.1 [10]. The European Medicines Agency (EMA) guidance is more stringent with a clinical study initiation cut-off ≥ 0.02 [11]. Although the current guidances focus primarily on the uptake transporters in the kidney (OCT2 and organic anion transporters 1 and 3 [OAT1 and OAT3]), the EMA and a recent publication from the International Transporter Consortium (ITC) recommend extending these guidelines to include MATE-mediated drug interactions [12].

The current decision trees within the FDA and EMA guidances focus on evaluating DDIs of individual transporters (e.g., OCT2, OAT1), rather than a transporter family (e.g., OATs, OCTs, MATEs). With the exception of cimetidine and pyrimethamine, few drugs have been identified as clinically potent inhibitors of renal organic ion transporters, and no drugs are selective inhibitors of individual renal organic cation transporters. In addition, because the inhibitors that have been identified are either promiscuous and inhibit multiple transporter families (e.g. quinidine) or are more potent inhibitors of organic cation transporter 1 (OCT1), an organic cation transporter that is highly expressed in the liver (e.g. procainamide) [13–15], distinguishing the impact of renal organic cation transporters on the victim drug's pharmacokinetics has been difficult.

In this study, a strategic screen was implemented to identify probe inhibitors of renal organic cation transport that are selective and can inhibit transport at clinically relevant concentrations. Through *in vitro* assays we identified the histamine 2 antagonist, nizatidine, as a selective inhibitor of MATE2K-mediated transport with an I_{fu}/IC_{50} ratio ≥ 0.1. To determine the clinical impact of MATE2K-selective inhibition on the pharmacokinetics and pharmacodynamics of a victim organic cation, an open-label, randomized, two-phase crossover DDI study was conducted using metformin as the victim drug and nizatidine as the perpetrator. Metformin is eliminated unchanged by renal mechanisms and while the liver is important for the pharmacologic activity of metformin, biliary excretion of metformin is negligible [16, 17]. The hypotheses of this clinical DDI study were (*i*) the co-administration of metformin and nizatidine will reduce the renal clearance (CL_R) of metformin, increase metformin concentration in the kidney and potentially lead to increased plasma concentrations and (*ii*) the interaction will enhance the hypoglycemic activity of metformin.

2 Methods

2.1 Physicochemical properties and clinical concentration of test inhibitors

Predicted charge at pH 7.4 was calculated using MarvinView Version 5.3.6 (ChemAxon, http://www.chemaxon.com). C_{max} values of test inhibitors in human subjects and percent protein binding in human plasma were obtained from literature sources (Supplemental Table 1; [18, 19]). $C_{max,u}$ values were calculated by $C_{max,u} = C_{max} \cdot f_u$, where f_u is the unbound fraction in human plasma.

2.2 *In vitro* identification of renal transporter inhibitors

2.2.1 Cell lines—Flp-In human embryonic kidney (HEK293-Flp-In) cells stably expressing human OCT1 (HEK-OCT1), OCT2 (HEK-OCT2), OCT3 (HEK-OCT3), MATE1 (HEK-MATE1), MATE2K (HEK-MATE2K) and the pcDNA5/FRT empty vector (HEK-EV) were previously established in our laboratory [8, 20–26]. Madin–Darby canine kidney type II (MDCK-II) cells stably expressing human PMAT (MDCK-PMAT) and the pcDNA3.1(+) vector (MDCK-EV) were also established previously [27].

2.2.2 Cellular Uptake Study—Cells were suspended in cell culture medium, seeded on poly-D-lysine-coated 48-well plates (Greiner Bio-One, Monroe, NC) and grown to ~90% confluency (~48 hours post seeding). Immediately prior to uptake, HEK-EV, HEK-OCT1,

HEK-OCT2 and HEK-OCT3 cells were preincubated for 20 min with Hank's balanced salt solution (HBSS). HEK-EV, HEK-MATE1 and HEK-MATE2K cells were preincubated with HBSS plus 30 mM NH_4Cl for 20 mins. MDCK-EV and MDCK-PMAT cells were preincubated with Krebs-Ringer-Henseleit (KRH) Buffer for 20 min. Preincubation media was removed and uptake was initiated with the addition of uptake buffer (HBSS [HEK cell lines] or KRH [MDCK cell lines] containing [^{14}C]-metformin [10 μM] with and without a test inhibitor at its 10x $C_{max,u}$) at 37°C for a period of time for which linear uptake of metformin was observed (2–5 min). At the end of the uptake, cells were washed twice with ice-cold buffer (HBSS [HEK cell lines] or KRH [MDCK cell lines]) and lysed with 0.1 N NaOH/0.1% SDS. Intracellular radioactivity was determined by liquid scintillation counting and normalized per well of protein content as measured by bicinchoninic acid protein assay (Thermo Scientific, Rockford, IL). Each test condition was conducted in triplicate. Compounds that were selective inhibitors of the renal organic cation transporters at their 10x $C_{max,u}$ were subjected to experimental IC_{50} determination. Studies were conducted exactly as described above using increasing concentrations of the inhibitor (ranging from 0–40x $C_{max,u}$ of the inhibitor for a total of 8 concentrations).

2.2.3 Determination of IC_{50} Values—After adjusting for protein quantity and subtracting non-specific transport of metformin (measured from empty vector cells), residual values were normalized to the rate of uptake in the absence of the inhibitor (set at 100%). Dose response curves and IC_{50} values were obtained using GraphPad Prism 4.0 (GraphPad Software, San Diego, CA). Briefly, inhibitor concentrations were transformed to log scale and dose-response inhibition curves were fitted with the following equation:

$$\%\,\text{metformin uptake} = \text{bottom} + (100 - \text{bottom})/(1 + 10^{\text{logIC50} - \log[\text{inhibitor}] * \text{hill coefficient}}),$$

where bottom is the plateau of maximum inhibition observed.

Absolute IC_{50} values were calculated by interpolation of the fitted curve.

2.3 Clinical Drug-Drug Interaction Study in Healthy Volunteers

2.3.1 Study Design—This was an open-label, randomized, two-treatment crossover study conducted in healthy subjects (n=12) at the University of California, San Francisco (UCSF) Clinical and Translational Science Institute Clinical Resources Services, San Francisco General Hospital (SFGH) Clinical site. To be eligible for the study, subjects had to provide written informed consent, be between the ages of 18 and 45 years, be in good health (as evidenced by medical histories, physical examination and routine clinical laboratory evaluations), not be on any medications other than oral contraceptives and not have any known allergies to iodine. Once enrolled, volunteers were directed to follow a controlled carbohydrate diet (200–250 g/day) 3 days prior to each inpatient visit. Subjects were admitted to the clinical facility the night before the first dose and remained on site for the duration of the study (36 hours). After an overnight fast (10 hours), study participants received either (*i*) an 850 mg oral dose of metformin (Glucophage®) or (*ii*) a simultaneous 850 mg oral dose of metformin and a 600 mg oral dose of nizatidine (Axid®). Each study participant received both treatments separated by a minimum of 7 days. A 3-hour oral

glucose tolerance test (OGTT, 75 g) was conducted 2 hours after metformin dosing (with or without nizatidine). Baseline OGTT's, without metformin, were obtained from the same subjects in a previous study [9]. Standardized meals were provided during inpatient visits after completion of the OGTT. Following the metformin dose, subjects were asked to drink 8 oz of water every 2 hours to maintain urine flow and pH. At 10 hours after metformin dosing, 1500 mg of iohexol (Omnipaque®) was administered by slow IV push over 5 minutes. Frequent timed blood samples were collected up to 24 hours post dosing to determine plasma metformin and nizatidine concentrations. Additional blood samples were collected 10 to 14 hours post metformin dosing to determine clearance of iohexol (a measure of glomerular filtration rate). For metformin pharmacodynamics, frequent blood samples were collected 0 to 180 minutes after glucose administration. An additional blood sample was collected at 12 hours after the second dose of metformin to determine serum creatinine concentrations. Urine samples were collected 0–2, 2–4, 4–8, 8–12 and 12–24 hours after metformin dosing to calculate metformin and creatinine CL_R.

2.3.2 Bioanalytical Methods—Metformin concentrations in plasma and urine were assayed by a validated liquid chromatography-tandem mass spectrometry method [28]. Nizatidine plasma concentrations were measured using the transitions *m/z* 332.29 to 58.08. Both the intra-day and inter-day coefficients of analysis variation were <5%. Iohexol concentrations in plasma were measured by University of Minnesota Physicians Outreach Labs (Minneapolis, MN). Lactate and glucose concentrations in plasma were analyzed by ARUP laboratories (Salt Lake City, UT). Creatinine concentrations in plasma and urine were measured by the clinical laboratories of SFGH using standard methodologies.

2.3.3 Data Analysis

2.3.3.1 Clinical Pharmacokinetics: The concentration-time curves of metformin and nizatidine were plotted using GraphPad Prism 4.0 (GraphPad Software, San Diego, CA). The pharmacokinetic parameters of metformin, nizatidine and iohexol were determined by non-compartmental analysis (WinNonlin 4.1, Pharsight Corporation, Mountain View, CA).

2.3.3.2 Statistical Analysis: Using previously reported metformin pharmacokinetic data in healthy volunteers [29], a sample size of 12 was needed to detect a 30% difference in metformin's renal clearance between the two treatments with >80% power. Data are presented as mean ± SD unless indicated otherwise. Paired nonparametric Student's *t*-tests were used to analyze the differences in metformin pharmacokinetic and pharmacodynamic parameters using GraphPad Prism 4.0. A statistically significant result was defined when $p < 0.05$.

3 Results

3.1 Identification of Drugs That Selectively Inhibit Renal Organic Cation Transporters at Clinically Relevant Concentrations

FDA-approved medications (n=71, Supplemental Table 1) were selected to be screened as potential inhibitors of MATE1, MATE2K and OCT2, if they met one or more of the following criteria: (*i*) C_{max} in human subjects was greater than 0.1 μM, (*ii*) f_u was greater

than 0.10 and (iii) predicted to have a positive or neutral charge at physiological pH (pH 7.4). These criteria were used to maximize the chance of identifying a clinically potent inhibitor of renal organic cation transporters. The selected drugs (Supplemental Table 1) span across various therapeutic classes (e.g., antibiotic, antiulcer, antiarrhythmic, antiemetic). Of the 71 drugs, which were screened at different concentrations reflecting their clinical concentrations, 4 (naloxone, quinine, procainamide and praziquantel) were inhibitors of OCT2, 10 (trimethoprim, cimetidine, ranitidine, moxifloxacin, chlorphenesin, quinine, clofazimine, abacavir, norfloxacin and ondansetron) were inhibitors of MATE1 and 11 (trimethoprim, chlorphenesin, cimetidine, ranitidine, moxifloxacin, norfloxacin, procainamide, ondansetron, famciclovir, nizatidine and quinine) were inhibitors of MATE2K, with ≥ 50% inhibition of [^{14}C]-metformin uptake at 10x $C_{max,u}$ (Supplemental Figure 1a–c).

Based on the OCT2, MATE1 and MATE2K screening results, inhibitors were grouped as follows: (*i*) OCT2-selective (naloxone and praziquantel), (*ii*) MATE1-selective (abacavir and clofazimine), (*iii*) MATE2K-selective (famciclovir and nizatidine), (*iv*) MATE1/MATE2K dual inhibitors (chlorphenesin, cimetidine, moxifloxacin, norfloxacin, ondansetron, ranitidine and trimethoprim) and (*v*) OCT2/apical inhibitors (procainamide and quinine). These compounds (n=15) were then assessed for their potential to inhibit OCT1 and OCT3, which are not thought to play a significant role in renal drug elimination. Of the 15 compounds, moxifloxacin, norfloxacin, ondansetron, ranitidine and nizatidine demonstrated minimal inhibition of OCT1- or OCT3-mediated metformin uptake at their 10x $C_{max,u}$ (metformin uptake was >50%) and were therefore designated as selective inhibitors of the renal organic cation transporters (Supplemental Figure 2a–e).

Follow-up IC_{50} studies of moxifloxacin, norfloxacin, ondansetron, ranitidine and nizatidine were conducted in cell lines expressing each of the known metformin transporters (OCT1, OCT2, OCT3, MATE1, MATE2K and PMAT) (Figure 1a–e, Table 1). Of note, moxifloxacin, norfloxacin and ondansetron, were identified as potential clinical inhibitors of both MATE1 ($C_{max,u}/IC_{50}$ is 0.74, 0.20 and 0.62, respectively) and MATE2K ($C_{max,u}/IC_{50}$ is 3.16, 0.84 and 0.39, respectively), but not of any other metformin transporters tested. In addition, nizatidine was identified as a potent and selective inhibitor of MATE2K ($C_{max,u}/IC_{50}$=0.37), but not of any other metformin transporters tested. Although ranitidine was a potent inhibitor of MATE1 and MATE2K-mediated transport, it did not demonstrate selective inhibition of the two MATE transporters. In all cases, the Hill coefficient was greater than one, suggesting positive cooperativity where more than one binding site is involved in the inhibition. However, additional kinetic analyses are warranted to confirm this observation.

3.2 Determination of the Clinical Impact of MATE2K Selective Inhibition on the Pharmacokinetics and Response Of Metformin in Healthy Volunteers

An open-label, randomized, two-phase crossover DDI study was conducted in healthy volunteers (n=12) to determine the effect of MATE2K-selective inhibition by nizatidine on the exposure and response of metformin. All subjects completed the study and no adverse

events were reported. The demographic and baseline characteristics of the healthy volunteers are shown in Table 2.

To maximize the potential for a clinical impact of MATE2K inhibition by nizatidine, a 600 mg oral dose of nizatidine (the maximum recommended daily dose) was administered to healthy volunteers. To verify that this dose of nizatidine achieved sufficient concentrations predicted to alter MATE2K activity, the pharmacokinetics of nizatidine was determined (Figure 2, Supplemental Table 2). Nizatidine reached a C_{max} of 4.2 ± 0.3 μg/mL (12.7 μM) and with 35% protein binding [19], its calculated $C_{max,u}$ was 2.7 ± 0.2 μg/mL (8.2 μM). This value is greater than the *in vitro* MATE2K inhibition potency (IC_{50}=7.8 μM) and above the recommended cut-off for conducting a clinical DDI study ($C_{max,u}/IC_{50}$=1.1) [11, 12].

Plasma concentration-time profiles for metformin were similar following administration of metformin alone or with nizatidine (Figure 3). When nizatidine was co-administered, the V/F (apparent volume of distribution) and $t_{1/2}$ (half-life) of metformin increased slightly, but significantly, by 38% and 24%, respectively ($p<0.05$, Table 3, Figure 4). Co-administration of nizatidine had no significant effect on metformin t_{max} (time to the maximal plasma concentration), C_{max}, $AUC_{0–24}$ (area under the concentration-time curve from 0–24 h time point), AUC_{inf} (AUC from 0 to infinity), and CL/F (apparent oral clearance) (Figure 3, Table 3). Importantly, no effects of nizatidine on the CL_R or CL_{SR} (net renal clearance by secretion) of metformin were observed in the 11 subjects with complete urine collection.

A rare, but serious adverse effect associated with metformin is lactic acidosis [30], where plasma lactate levels exceed 5 mM. There was no significant difference in plasma lactate concentrations between treatment arms (data not shown) and observed maximum concentrations were below the 5 mM toxicity threshold in both treatment groups (metformin alone, 1.7 ± 0.1 mM; metformin with nizatidine, 1.6 ± 0.1 mM).

The glucose-lowering effect of metformin was determined in healthy subjects by administering a 3-hour OGTT (Supplemental Figure 3). We observed similar glucose AUC values after the OGTT between pre-metformin and metformin alone treatment periods (pre-metformin, 3298 ± 145 mg hr/L; metformin alone, 3100 ± 121 mg hr/L; $p>0.1$; Figure 5). However, after metformin was co-administered with nizatidine, there was a significantly lower glucose AUC (greater response) compared to pre-metformin values (pre-metformin, 3298 ± 145 mg hr/L; metformin with nizatidine, 3015 ± 74 mg hr/L; $p=0.03$; Figure 5).

4 Discussion

Historically, DDIs were thought to occur primarily by interactions with drug-metabolizing enzymes. Current evidence suggests a role for drug transporters in mediating clinical DDIs [31, 32], however transporter-mediated DDIs have been less well characterized. As noted previously, few inhibitors of renal organic cation transporters have been identified and, of these inhibitors, none has been shown to selectively inhibit a single transporter involved in the renal elimination of basic drugs. Further, previous clinical investigations of renal organic cation transporter-mediated DDIs have focused on drugs that are predicted to alter the activity of more than one transporter [1–4, 33–36]. Thus, there is a poor understanding of the mechanisms involved in the renal clearance of organic cations in general, and of

metformin in particular. Through a strategic *in vitro* screen, nizatidine was identified as a clinically potent and selective inhibitor of MATE2K mediated uptake of metformin. Thus, nizatidine represents a useful tool to understand the clinical significance of MATE2K in the renal elimination of metformin. In this study, nizatidine increased the apparent volume of distribution and hypoglycemic activity of metformin. However, despite achieving unbound maximum concentrations that exceeded the *in vitro* inhibition potency of MATE2K-mediated transport, nizatidine did not alter the renal clearance or net secretory clearance of metformin.

In our in vitro screen, several drugs appeared to stimulate metformin uptake in OCT2, MATE1 and MATE2K-transfected cells. This enhancement of *in vitro* activity has also been observed for a variety of solute carrier and ATP-binding cassette transporters including OAT1, MRP2, OATP1B1/1B3, and OCT2 [37–41]. Previous reports have observed the dependence of OCT2-mediated transport on inside-negative membrane potential [42, 43]. Thus, alterations in membrane potential by test compounds may explain an enhancement in metformin uptake. In our data set, the enhancement in metformin uptake was more common in MATE-transfected cell lines, which are particularly affected by alterations in pH as they transport organic cations in exchange for a proton [5, 44, 45]. Therefore, it is possible that the test compounds could have altered the pH gradient, leading to an enhancement in metformin uptake by MATE-transfected cells. Other possibilities include positive cooperativity of compounds with the transporters as well as effects of compounds on potential difference across intracellular organelles (e.g., mitochondria).

Several reasons may be proposed to explain the lack of effect of nizatidine on metformin CL_R. First, previous DDI studies examining the effect of perpetrator drugs on metformin CL_R have used non-selective inhibitors of MATE1 and MATE2K. For example, in a previous clinical DDI study, there was a significant reduction in the CL_R and CL_{SR} of metformin after co-administration of a MATE1/MATE2K dual inhibitor, pyrimethamine [2]. However, in the current study, we did not observe a significant reduction in the CL_R or CL_{SR} of metformin when co-administered with nizatidine, a MATE2K selective inhibitor. Compared to pyrimethamine, the half-life of nizatidine is much shorter (pyrimethamine $t_{1/2}$, ~4 days [19]; nizatidine $t_{1/2}$, ~3 hours). It is probable that the duration of inhibition is an important determinant of alterations in CL_R, which is generally estimated over a 24-hour period. However, we also examined the fractional CL_R and CL_{SR} (e.g. 0–2 hour, 2–4 hour, 0–4 hour) of metformin, and found no differences between treatment arms (data not shown). Thus, the selective inhibition of MATE2K may be insufficient to have a measurable effect on the CL_R and CL_{SR} of metformin.

A second reason for the lack of reduction in metformin renal clearance by nizatidine is that metformin may cross the luminal membrane of the proximal tubule cell primarily by MATE1, and that MATE2K has a limited role. However, studies demonstrating an association between genetic variants of MATE2K and metformin CL_R suggest that MATE2K does play a role in the renal elimination of metformin [8, 9, 46]. It is also possible that the *in vitro* methods used in this study incorrectly predicted the concentrations of nizatidine required to inhibit MATE2K *in vivo*. The IC_{50} for nizatidine on MATE2K-mediated transport was determined *in vitro* using a proton-driven influx of metformin.

However, *in vivo* MATE2K functions as an efflux pump. A previous study examining MATE1 transport of MPP^+ (1-methyl-4-phenylpyridinium) demonstrated that there are symmetrical interactions of H^+ with inward-facing and outward-facing MATE1 [47]. This has not been confirmed for MATE2K-mediated transport of metformin. However, it should be noted that for *in vitro* studies of both pyrimethamine and cimetidine, MATE1/2K dual inhibitors of metformin transport, influx methods were used to predict IC_{50} values [2, 15].

Other possibilities are that concentrations of nizatidine in the renal tubule or tubule cells were not sufficient to inhibit the transporter. However, nizatidine had no effect on fractional CL_R of metformin at earlier times, when nizatidine concentrations were higher. Notably, metformin concentrations were also higher at these earlier times; thus, nizatidine concentrations may not have been sufficient to reduce metformin CL_R. Though speculative, it is also possible that nizatidine inhibited transporters involved in metformin reabsorption that counteracted its effects on CL_{SR}. Because nizatidine is actively secreted in the kidney, its concentrations in the proximal tubule and proximal tubule cells should be higher than its plasma unbound concentrations [48].

These results suggest that further studies are needed to inform current guidelines provided by the EMA [11]. The EMA suggests that $I_{fu}/IC_{50} \geq 0.02$, should trigger consideration of a clinical DDI study. However, our data suggest that very selective inhibitors may not be sufficient to cause measurable DDIs with organic cation transporters in the kidney, or that threshold ratios between I_{fu} and IC_{50} for selective inhibitors should be higher to trigger consideration of a clinical study. More promiscuous inhibitors such as pyrimethamine and cimetidine clearly result in clinical DDIs and current ratios to trigger consideration of a clinical study, though conservative, are acceptable for these non-selective inhibitors. However, our data with nizatidine suggest that a MATE2K selective inhibitor, which achieved concentrations above its IC_{50}, clearly did not result in a clinical DDI, and that different threshold ratios for triggering consideration of a clinical DDI may be warranted.

In this study, we observed a significant increase in metformin V/F and a related increase in metformin $t_{1/2}$. These results are consistent with previous reports that inhibitors of renal transporters have a direct effect on the victim drug's volume of distribution [49]. As MATE2K is highly expressed in the kidney, an increase in metformin V/F suggests that metformin may accumulate in the renal cell when its MATE2K-mediated efflux is blocked by nizatidine co-administration.

Interestingly, while we did not see any alterations in systemic levels of metformin, the hypoglycemic activity (as determined by glucose AUC) was enhanced above pre-metformin values only when nizatidine was co-administered. Although not determined in the study, nizatidine is not known to alter glucose levels [50]. While the kidney is mostly known for its role in the reabsorption of glucose from the filtrate, it also has a significant impact on glucose uptake from blood (20% of total uptake in the post-absorptive state) [51], gluconeogenesis (20–25% of total glucose release in the post-absorptive state) [52–54] and glucose utilization (post-absorptive, 5–10%; post-prandial, 10–15% of total) [55, 56]. In the liver, metformin is known to enhance glucose uptake, decrease glucose production and increase glucose utilization (for review see [57]). Consistent with the increase in metformin

V/F, our data suggest that nizatidine increases metformin levels in the kidney where it is then able to enhance glucose uptake and utilization. Our data demonstrating that inhibition of MATE2K increases response to metformin in healthy subjects are consistent with previous studies from our laboratory demonstrating a reduced response to metformin in type 2 diabetic patients who are carriers of an allele of MATE2K that is associated with increased transcription rates of the transporter [8, 9]. The concept that increasing metformin levels in the liver or kidney through inhibition of MATEs enhances its pharmacologic effects needs further study. Similar concepts have been shown for renal toxicity to platinums [58], i.e., inhibition of MATEs enhances platinum accumulation in the kidney resulting in increased nephrotoxicity [6, 7]. Further, mice with Mate1 deficiency exhibit increased metformin-induced lactic acidosis [59].

This study raises questions about extrapolating *in vitro* inhibition IC_{50} data to clinical DDI studies, and suggests that further refinement of current FDA and EMA guidelines are needed. Those refinements may include recommendations related to measurements of intracellular drug levels, relative half-life of the inhibitor compared with the substrate, and selectivity of the inhibitor for renal transporters. Clearly, future studies are needed to define best practices and standardize *in vitro* methodologies that accurately predict clinical DDIs and to understand the effects of selective versus nonselective inhibitors on renal drug elimination.

5 Conclusions

In healthy volunteers, a MATE2K-selective inhibitor, nizatidine, increased the apparent volume of distribution and hypoglycemic activity of metformin. However, despite achieving unbound maximum concentrations that were greater than the *in vitro* inhibition potency of MATE2K-mediated transport, nizatidine did not change metformin's renal clearance or net secretory clearance. This study highlights that current guidelines, which rely on *in vitro* predictions to inform the decision to conduct transporter-mediated clinical DDI studies, need refinement [10–12]. Further, this study suggests that physiologically based pharmacokinetic models to predict intracellular and intra-tubular concentrations of drugs should be developed and used in combination with *in vitro* data to predict DDIs in the kidney. Finally, this study supports previous studies highlighting that inhibition of efflux transporters will have important effects on the apparent volume of distribution, tissue levels and peripheral effects of victim drugs [49].

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Key Points

1. Nizatidine was identified as a selective *in vitro* inhibitor of MATE2K-mediated transport of metformin at clinically relevant concentrations.
2. In healthy subjects, nizatidine increased the apparent volume of distribution and hypoglycemic activity of metformin. However, despite achieving unbound maximum concentrations that exceeded the *in vitro* inhibition potency of MATE2K-mediated transport, nizatidine did not alter the renal clearance or net secretory clearance of metformin.
3. The data from this study demonstrates that very selective inhibitors may not be sufficient to cause measurable DDIs with organic cation transporters in the kidney. In addition, this study challenges current health authority guidelines that rely on *in vitro* predictions to inform the decision to conduct transporter-mediated clinical DDI studies.

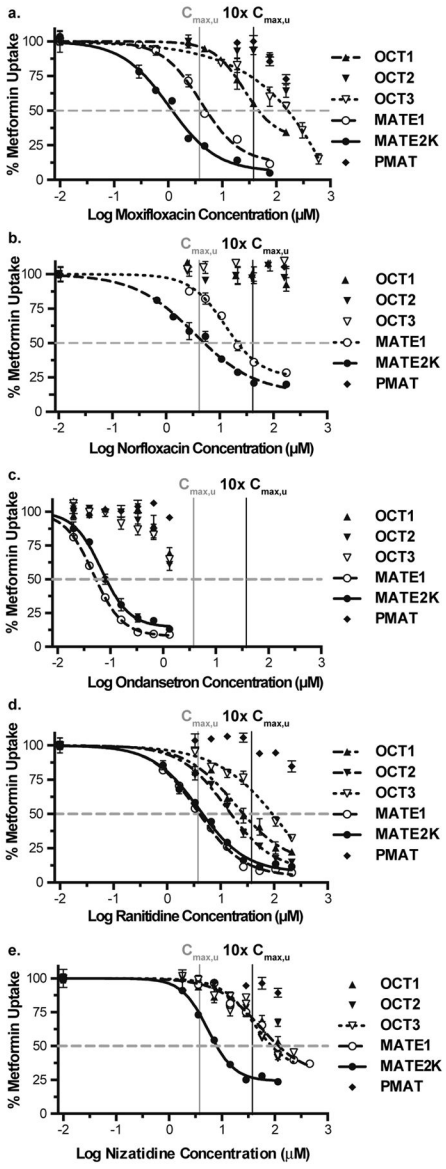

Figure 1. Inhibitory potency of various drugs on metformin uptake in cell lines expressing organic cation transporters

Shown are five compounds that potently inhibited MATE2K in the screening studies at or below therapeutically relevant unbound concentrations. Increasing concentrations of (a) moxifloxacin, (b) norfloxacin, (c) ondansetron, (d) ranitidine and (e) nizatidine were analyzed for their ability to inhibit metformin uptake in cells stably overexpressing OCT1, OCT2, OCT3, MATE1, MATE2K and PMAT. IC_{50} values were determined for drugs/cell lines that display ≥50% inhibition of metformin uptake (horizontal red line). The vertical grey and black lines are drawn at 1x and 10x the inhibitor's $C_{max,u}$, respectively. Values are presented as mean ± SEM (n = 3).

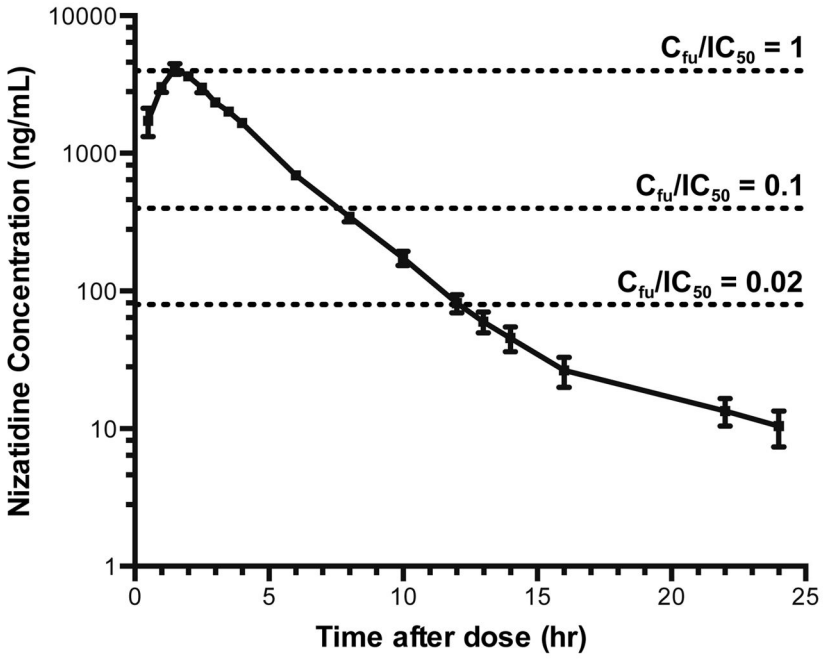

Figure 2. Mean nizatidine plasma concentrations following administration of a single oral dose to 12 healthy volunteers

Nizatidine plasma concentrations were determined after a 600 mg single oral dose in combination with metformin. The dotted horizontal lines are drawn at C_{fu}/IC_{50} ratios of 1, 0.1 (FDA cut-off) and 0.02 (EMA cut-off). Data represent mean ± SEM.

C_{fu}, unbound concentration in plasma; IC_{50}, concentration at half the maximum inhibition of active transport; FDA, Food and Drug Administration; EMA, European Medicines Agency

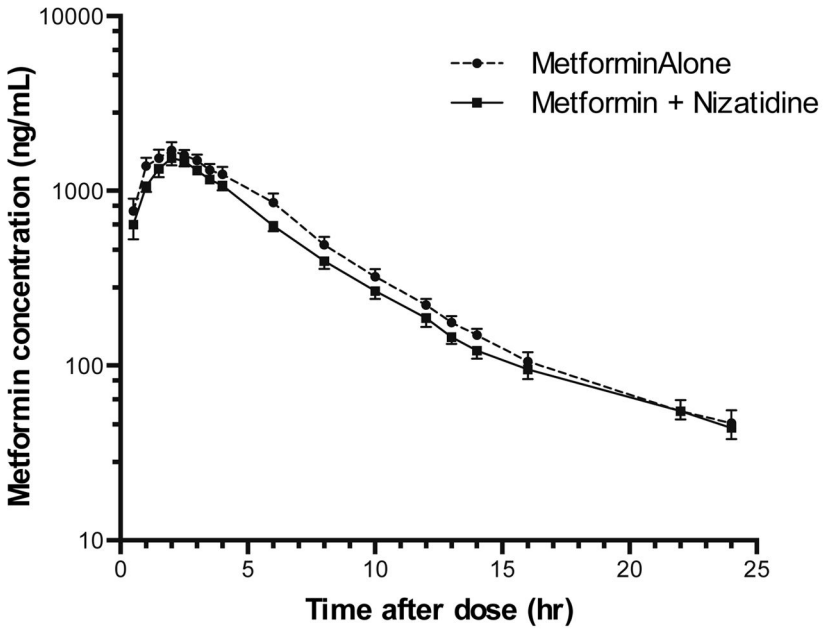

Figure 3. Mean metformin plasma concentration-time curves after administration of metformin alone or with nizatidine to 12 healthy volunteers

Metformin plasma concentrations were determined after a single oral dose (850 mg) alone (blue) or in combination with a single oral dose (600 mg) of nizatidine (red). Data represent mean ± SEM.

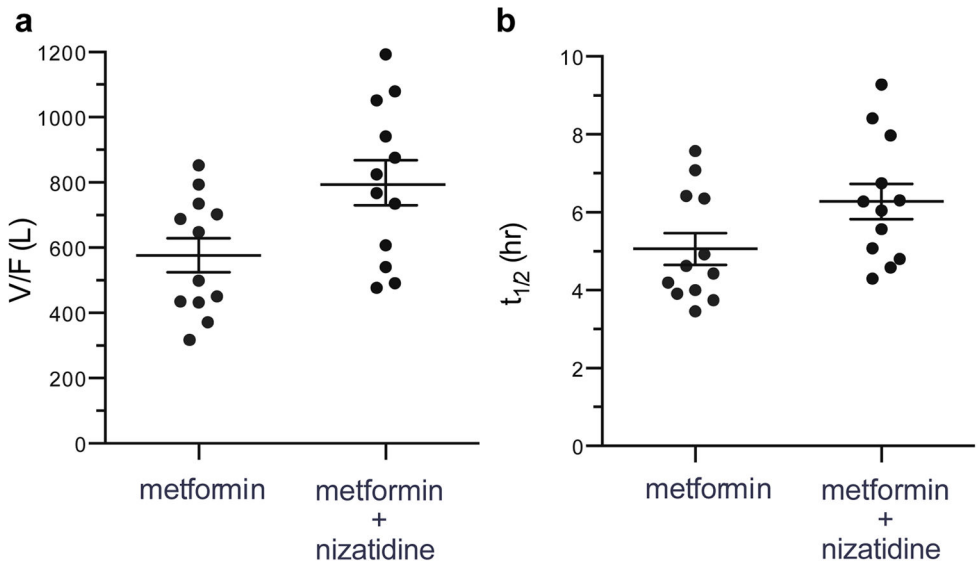

Figure 4. Volume of distribution (a) and half-life (b) alterations between treatment groups

Symbols, horizontal line and brackets represent individual data points, mean value of all subjects and standard error of the mean, respectively.

V/F, apparent volume of distribution; $t_{1/2}$, plasma terminal elimination half-life.

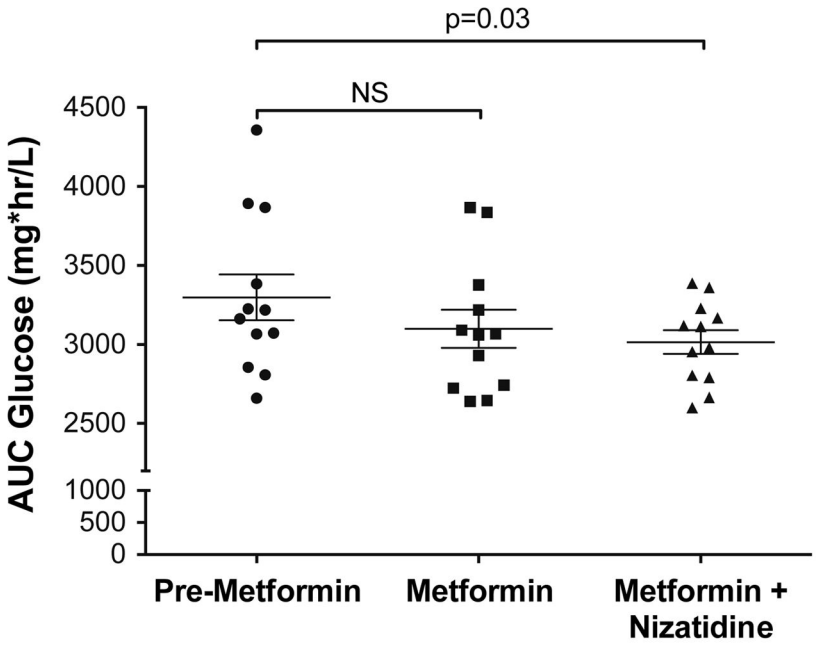

Figure 5. The effect of nizatidine on plasma glucose concentrations after metformin treatment

The area under the plasma glucose concentration-time curve (AUC glucose) was calculated during the oral glucose tolerance test. Symbols, horizontal line and brackets represent individual data points, mean value of all subjects and standard error of the mean, respectively. NS, not significant.

Table 1

In vitro potency of putative clinical inhibitors in cells expressing OCT1, OCT2, OCT3, MATE1, MATE2K and PMAT.

Drug	Parameter	OCT1	OCT2	OCT3	MATE1	MATE2K	PMAT
moxifloxacin	**IC_{50} (μM)**	47.0	>151	>151	5.12	1.19	>151
	C_{max}/IC_{50}	**0.13**	<0.04	<0.04	**1.22**	**5.22**	<0.04
	$C_{max,u}/IC_{50}$	0.08	<0.02	<0.02	**0.74**	**3.16**	<0.02
norfloxacin	**IC_{50} (μM)**	>160	>160	>160	19.9	4.74	>160
	C_{max}/IC_{50}	<0.03	<0.03	<0.03	**0.24**	**0.99**	<0.03
	$C_{max,u}/IC_{50}$	<0.02	<0.02	<0.02	**0.20**	**0.84**	<0.02
ondansetron	**IC_{50} (μM)**	>1.32	>1.32	>1.32	0.05	0.08	>1.32
	C_{max}/IC_{50}	<0.10	<0.10	<0.10	**2.47**	**1.57**	<0.10
	$C_{max,u}/IC_{50}$	<0.02	<0.02	<0.02	**0.62**	**0.39**	<0.02
ranitidine	**IC_{50} (μM)**	26.8	15.7	93.5	4.04	4.63	>214
	C_{max}/IC_{50}	**0.24**	**0.40**	0.07	**1.56**	**1.36**	<0.03
	$C_{max,u}/IC_{50}$	**0.20**	**0.34**	0.06	**1.33**	**1.16**	<0.02
nizatidine	**IC_{50} (μM)**	>115	>115	41.1	52.7	7.81	>115
	C_{max}/IC_{50}	<0.04	<0.04	**0.11**	0.08	**0.56**	<0.04
	$C_{max,u}/IC_{50}$	<0.02	<0.02	0.07	0.05	**0.37**	<0.02

C_{max} and calculated $C_{max,u}$ were obtained from literature values (see Supplemental Table 1). C_{max}/IC_{50} and $C_{max,u}/IC_{50}$ values that exceed the 0.1 cut-off are bolded.

IC_{50}, concentration at half the maximum inhibition of active transport; C_{max}, maximum plasma concentration; $C_{max,u}$, maximum plasma concentration that is not bound to plasma proteins; OCT, organic cation transporter; MATE, multidrug and toxin extrusion transporter; PMAT, plasma membrane monoamine transporter.

Table 2

Demographic and baseline characteristics of healthy volunteers who participated in the clinical drug-drug interaction study between nizatidine and metformin

Characteristic	N = 12
Age (years)	
Mean (range)	27 (23 – 33)
Sex, n (%)	
Male	6 (50.0)
Female	6 (50.0)
Ethnicity, n (%)	
African American	6 (50.0)
Caucasian	3 (25.0)
Asian American	3 (25.0)
Weight (kg)	
Mean (range)	74 (49 – 101)
Height (cm)	
Mean (range)	176 (160 – 192)
BMI (kg/m^2)	
Mean (range)	23.9 (18.1 – 28.6)
eGFR (mL/min)	
Mean (range)	114 (89 – 143)

BMI, body mass index; eGFR, estimation of glomerular filtration rate as assessed by MDRD (Modification of Diet in Renal Disease) at screening using the African American correction factor when relevant.

Table 3

Summary of the pharmacokinetic parameters of metformin in healthy volunteers with and without nizatidine co-administration.

	Metformin Alone	Metformin + Nizatidine	p-value
t_{max} (hr)	2.46±0.66	2.13±0.38	0.09
C_{max} (μg/mL)	1.81±0.60	1.68±0.55	0.43
$AUC_{0–24}$ (μg.hr/mL)	11.0±3.1	9.6±2.1	0.20
AUC_{inf} (μg.hr/mL)	11.4±3.3	10.0±2.4	0.25
V/F (L)	577±179	799±239	0.01
CL/F (mL/min)	1360±440	1480±330	0.46
$t_{1/2}$ (hr)	5.1±1.4	6.3±1.6	0.05
[a] Amount in $urine_{0–24}$ (mg)	437±86	376±128	0.10
[a] CL_R (mL/min)	736±204	670±133	0.18
CL_{IHX} (mL/min)	118±19	110±13	0.09
[a] CL_{CR} (mL/min)	156±40	147±26	0.28
[a] CL_{SR} (mL/min)	615±201	558±134	0.22

[a] Due to incomplete urine collection from one subject, the amount in urine, CL_R, CL_{CR}, CL_{SR} were calculated from 11 subjects. Data is presented as mean ± SD.

t_{max}, time to the maximal plasma concentration; C_{max}, maximal plasma concentration; $AUC_{0–24}$, area under the concentration-time curve from 0–24 hr time point; AUC_{inf}, area under the concentration-time curve from 0 to infinity; V/F, apparent volume of distribution; CL/F, apparent oral clearance; $t_{1/2}$, plasma terminal elimination half-life; CL_R, renal clearance; CL_{IHX}, iohexol clearance, CL_{CR}, creatinine clearance; CL_{SR}, net renal clearance by secretion (metformin CL_R less CL_{IHX} for each individual subject)