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ORIGINAL ARTICLE

Comparative renal excretion of VX-702, a novel p38 MAPK inhibitor, and methotrexate in the perfused rat kidney model

Mitalee Tamhane[1], Ananthsrinivas R. Chakilam[2], Andrew Jayaraj[2], Vineet Thakkar[2] and David R. Taft[1]

[1]*Division of Pharmaceutical Sciences, Long Island University, Brooklyn, NY, USA and* [2]*Vertex Pharmaceuticals Inc., Cambridge, MA, USA*

Abstract

Context: VX-702 is a novel p38 mitogen-activated protein kinase inhibitor being developed to treat rheumatoid arthritis. *Objective*: To characterize the renal excretion profile of VX-702 using the isolated perfused rat kidney (IPRK) model. *Methods*: Studies were performed to assess the dose linearity of VX-702 excretion and to evaluate the effect of inhibitors of organic anion (probenecid) and organic cation (cimetidine) transport systems on VX-702 disposition. VX-702 excretion was studied over a range of doses targeting concentrations between 100 and 600 ng/mL. VX-702 (600 ng/mL) was also co-perfused with probenecid (1 mM) and cimetidine (2 mM). The results were compared to parallel experiments performed with methotrexate (MTX). *Results*: VX-702 excretion was linear over the range of doses studied, and clearance data were consistent with net reabsorption by the kidney. Transport inhibition studies indicate that VX-702 is not a substrate for renal organic anion and organic cation transport systems. MTX (500 ng/mL) also displayed net reabsorption in the IPRK, but secretory transport was inhibited upon co-administration with probenecid. This finding is consistent with previous IPRK studies that demonstrated inhibitory effects of NSAIDS on MTX excretion. *Conclusion*: Overall, this study suggests that a renal drug-drug interaction between VX-702 and MTX would be unlikely if these medications were co-administered.

key words: *Cimetidine; drug–drug interaction; probenecid; renal excretion; transport*

Introduction

Rheumatoid arthritis (RA) is a chronic autoimmune inflammatory disorder affecting an estimated 2.1 million people in the United States (http://ww2.arthritis.org/conditions/DiseaseCenter/RA/ra_who.asp, accessed February 18, 2009). RA is characterized by joint aches and stiffness, progressing to inflammation of the synovium (inner lining of the joints) and irreversible damage to cartilages, tendons, and bones. Although there is presently no cure available for RA, drug therapy aims to reduce disease progression, thereby supporting a healthy independent lifestyle for RA patients.

Treatment options for RA include medications to reduce inflammation such as nonsteroidal anti-inflammatory drugs (NSAIDs) and steroids. Diseases modifying antirheumatic drugs such as methotrexate (MTX) act directly on the immune system to halt disease progression. MTX is often prescribed in combination with NSAIDS. However, NSAIDs are associated with severe gastrointestinal side effects and cardiovascular safety concerns[1]. Additionally, NSAIDs are known to inhibit the renal secretion of MTX[2], and this drug–drug interaction has been associated with increased MTX exposure and toxicity in patients[3–5].

A new group of medications called biologic response modifiers have emerged as a therapeutic option for RA. Biologic response modifiers target the immune system and work by altering interaction of cytokines (signaling proteins that act as inflammatory mediators) with their

Address for correspondence: Dr. David R. Taft, Office of the Dean, Division of Pharmaceutical Sciences, Long Island University, 75 Dekalb Ave, Brooklyn, NY 11201, USA. E-mail: dtaft@liu.edu

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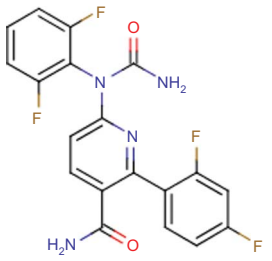

Figure 1. Chemical structure of VX-702. Obtained from http://kinasepro.wordpress.com.

receptors. Medications in this category include tumor necrosis factor alpha (TNFα) antagonists such as infliximab, etanercept, and adalimumab[6].

VX-702 (Figure 1) is a novel compound under development by Vertex Pharmaceutical Inc. (Cambridge, MA, USA). VX-702 is a highly selective inhibitor of the α isoform of p38 mitogen-activated protein kinase (MAPK) that binds to its ATP pocket and inhibits the kinase competitively. p38 MAPK is a major intracellular signaling pathway activated by inflammatory cytokines, bacterial and viral proteins such as lipopolysaccharides, heat, free radicals, and ultraviolet radiation[7,8]. Once activated, p38 MAPK modulates the expression of inflammatory mediators including cytokines [e.g., TNFα, interleukin 1β (IL-1β)], cyclooxygenase-2, and collagenase-1 and collagenase-3. The inflammatory cytokines, namely, TNFα and IL-1β are identified as key players in RA[8,9]. By blocking cell activation and production of proinflammatory cytokines, p38 MAPK inhibitors represent a new drug treatment for inflammatory diseases such as RA.

VX-702 has been studied in two 12-week, double-blind, placebo-controlled studies in patients with active, moderate to severe RA. In these studies, VX-702 showed a modest clinical efficacy plus a transient suppression of inflammatory biomarkers, suggesting that p38 MAPK inhibition may not provide meaningful, sustained suppression of the chronic inflammation seen in RA[10].

In this investigation, the renal excretion of VX-702 was studied in the isolated perfused rat kidney (IPRK) model. The IPRK is an established model for assessing renal mechanisms of elimination and screening for drug–drug interactions originating in the kidney[11,12]. Because VX-702 is primarily excreted by renal mechanisms, the aims of the study were to assess dose-linearity of VX-702 excretion and to probe renal transport mechanisms for this compound. An additional goal of the research was to compare the renal excretion profiles of VX-702 and MTX. Given the potential for combination therapy of these two medications in RA patients, it is important to determine whether they share a common renal transport pathway that could represent a site for a potentially toxic drug–drug interaction in the clinical setting.

Materials and methods

Chemicals

VX-702 and VRT-101793 were provided by Vertex Pharmaceuticals Incorporated. MTX, probenecid, cimetidine, bovine serum albumin (BSA), dextran, sodium chloride, sodium bicarbonate, calcium chloride, potassium chloride, magnesium sulfate, potassium phosphate, amino acids, inulin, glucose, anthrone, sulfuric acid (reagent grade), and water (HPLC grade) were purchased from Sigma Chemical Company (St. Louis, MO, USA).

Animals

Male Sprague–Dawley rats weighing approximately 300 g (Harlan, Indianapolis, IN, USA) were used for the experiments. The rats were housed in stainless steel cages and fed the standard chow and allowed free access to water. The Institutional Animal Care and Usage Committee of Long Island University approved the experimental protocol for these studies.

Isolated perfused kidney preparation

Before the surgery, the rats were anesthetized by intramuscular injection of ketamine HCl/xylazine HCl solution (800/120 mg, dose: 1 mL/kg). Surgery was performed according to the Bowman modification of the Nishiitsutsuji–Uwo procedure[13,14]. Briefly, a midline incision was made extending from the bladder to the sternum and the right ureter along with the right kidney was exposed. The right ureter was cannulated using a PE-10 tubing to facilitate urine collection during the experiment. The right renal artery was cannulated through the superior mesenteric artery using a 19G stainless steel needle, the other end of which was connected to the perfusion line through a three-way stopcock. Perfusion was commenced as soon as the needle was tied in place; the kidney was then excised and placed in the perfusion assembly.

Perfusion was performed as described by Bekersky[11]. Perfusate was composed of Krebs–Henseleit buffer (pH 7.4), 4% BSA, 1.67% dextran (oncotic agent), 100 mg/dL glucose, 60 mg/dL inulin [glomerular filtration rate (GFR) marker], and an amino acid solution (13 mM). The volume of the recirculating perfusate was 80 mL.

Study groups

Renal excretion of VX-702 was studied at three doses (8, 24, 48 µg) targeting initial perfusate concentrations of 100, 300, and 600 ng/mL. For transport inhibition

studies, VX-702 was co-perfused with probenecid [organic anion transport (OAT) inhibitor, 1 mM] or cimetidine [organic cation transport (OCT) inhibitor, 2 mM]. All experiments were performed in triplicate.

The renal excretion of MTX was evaluated at a clinically relevant plasma concentration (0.5 μg/mL) in the absence and presence of transport inhibitors (cimetidine and probenecid).

Experimental design

Each IPRK experiment was conducted for a period of 2 hours. Following kidney excision and transfer to the recirculating apparatus, the preparation was allowed to stabilize for 10 minutes. Next, a bolus dose of drug vehicle (control) or inhibitor (transport studies) was added to the perfusion reservoir. Following a 10-minute distribution period, test compound (VX-702 or MTX) was added as a bolus dose. Perfusate (0.8 mL) was sampled 5 minutes post-dose and every 10 minutes thereafter. Urine was collected at 10-minute intervals throughout the experiment.

Aliquots of perfusate and urine samples were analyzed for glucose and sodium using a Beckman CX-3 Clinical Chemistry Analyzer (Beckman-Coulter, Brea, CA, USA). Inulin was measured by a previously validated colorimetric assay[15].

Parameters monitored to assess kidney function included GFR, fractional reabsorption of glucose (FR_{GLU}) and sodium (FR_{Na}), urine flow rate, and urine pH. Perfusion pressure was maintained at 100±10 mmHg by adjusting perfusion flow rate as necessary.

Perfusate binding

Binding of VX-702 and MTX to perfusate was determined by ultrafiltration. VX-702 samples were prepared in various concentrations (50, 100, 250, 500, and 600 ng/mL) in the absence and presence of probenecid or cimetidine. Parallel experiments were performed with MTX (500 ng/mL). Samples (2 mL) were equilibrated in a shaker bath (37°C) for an hour, and an aliquot (0.5 mL) was collected for determination of total drug concentration. A second aliquot (1 mL) was transferred to an Amicon Centrifree Micropartition System (Millipore Corporation, Bedford, MA, USA) and centrifuged at 1500 × *g* for 15 minutes. The resultant ultrafiltrate was then used to determine free drug concentration. Preliminary experiments were performed to assess the binding of each compound under study, to the ultrafiltrate device membrane, and it was found that the binding was insignificant. The fraction of drug unbound in perfusate (f_u) was estimated as the ratio of unbound and total concentrations.

Sample analysis

VX-702 was quantified using a liquid chromatography with mass spectrometry (LC–MS/MS) method. VRT-101793 was used as an internal standard. Samples (100 μL) were pipetted into clean 96 deep well plates. One hundred microliters of VRT-101793 and 100 μL of standard sodium chloride solution were added, and the samples were vortexed for 60 seconds. Four hundred microliters of methyl-tertiary butyl ether was added, and the mixture vortexed (60 seconds) and centrifuged (1500 × *g*) for 10 minutes. Two hundred and seventy-five microliters of organic layer was transferred to a clean 96-well block, and the samples were evaporated to dryness under nitrogen. Dried extract was reconstituted with 100 μL of mobile phase, vortexed for 3 minutes, and centrifuged at 1500 × *g* for 8 minutes. Ten microliters of sample was injected onto 5.0 μm Symmetry C-18 reverse phase column (4.6 × 50 mm). The mobile phase used was a mixture of 0.1% formic acid in water and 0.1% formic acid in acetonitrile (60:40, v/v). The flow rate was 0.8 mL/min. The column elute was determined by positive ion mode using the turbo ion spray (ESI^+) interface MS/MS detection (Sciex API 4000 Q trap). The lower and upper limits of quantification were 0.5 and 800 ng/mL, respectively.

MTX was also analyzed using LC–MS/MS. One hundred microliters of sample was pipetted into clean 96 deep well plates. Exactly 100 μL of 100 ng/mL of D3-MTX (internal standard) was added to the samples, vortexed for 120 seconds, and centrifuged at 1500 × *g* for 20 minutes. Four hundred microliters of methanol was added as extraction solvent. After processing, the solvent was evaporated using nitrogen and the dried extract reconstituted with 0.1% formic acid in water, vortexed for 2 minutes, and centrifuged at 1500 × *g* for 10 minutes. One hundred microliters of sample was transferred to injection plates and an aliquot (20 μL) was injected into 5.0 μm Xterra reverse phase C18 MS column (2.1 × 50 mm). The mobile phase was composed of 0.1% formic acid in water:acetonitrile:methanol (80:15:15). The flow rate was 0.25 mL/min. The column elute was analyzed by positive ion mode using the turbo ion spray (ESI^+) interface MS/MS detection (Sciex API 4000 Q trap). For MTX, the lower limit of quantification was 2 ng/mL.

Data analysis

Renal clearance during each collection period was calculated using the following equation:

$$Cl = \frac{\mathrm{UFR} \times C_{\mathrm{urine}}}{C_{\mathrm{perfusate}}}, \quad (1)$$

where UFR represents the urine flow rate, C_{urine} represents the concentration of analyte (VX-702 or MTX) in urine, and $C_{perfusate}$ represents the concentration of analyte in the perfusate at the midpoint of the urine collection period. GFR was estimated as inulin clearance (using Equation 1).

Renal excretion ratio (XR), an indication of net mechanisms of elimination, was calculated as the ratio of clearance to filtration clearance (GFR $\times f_u$). XR values greater than 1 indicate net tubular secretion by the kidney. XR values less than 1 are consistent with net tubular reabsorption[16].

Statistical analysis

All data were expressed as mean ± SD. One-way analysis of variance (ANOVA) was used to compare the mean parameter estimates of IPRK viability criteria for control and drug treatment groups. Dunnett's test was used to determine the study groups that differed from control regarding viability criteria. ANOVA was also utilized to compare the differences in renal excretion parameters. Post hoc analysis was used to indicate the effect of increasing dose on renal excretion of VX-702 as well as determine the effect of concurrent administration of transport inhibitors on drug (VX-702 or MTX) excretion.

Results

Functionality of the IPRK

Table 1 summarizes the IPRK viability data across experimental treatment groups. Based on control (drug-naive) studies, renal function was well maintained in this investigation. Viability parameters were within acceptable limits and are consistent with published IPRK data[11–13]. Despite statistically significant differences noted between study groups and control, these differences were not physiologically significant.

VX-702 excretion in the IPRK: dose-linearity studies

Table 2 contains renal excretion parameters for VX-702 in the IPRK. Over the range of concentrations studied, VX-702 displayed linear renal excretion. Although differences in clearance were noted among treatment groups, these differences were attributed to inter-kidney variations in GFR as renal clearance reflects the net contributions of glomerular filtration, tubular secretion, and tubular reabsorption. Hence, differences in GFR among individual perfusion experiments would be expected to influence the estimate of clearance in the IPRK model. As seen in the table, XR (a parameter that corrects for GFR differences) was consistent among the doses studied. Perfusate binding of VX-702 was approximately 27% ($f_u \sim 0.7$).

VX-702 excretion in the IPRK: transport inhibition studies

Renal excretion parameters for VX-702 in the presence of probenecid and cimetidine are presented in Table 3. Co-administration of these transport inhibitors had no apparent effect on VX-702 renal excretion in the IPRK. As with dose-linearity studies, differences in clearance noted among treatment groups were attributed to inter-kidney differences in GFR. Co-administration of the interactants did not affect VX-702 protein binding.

MTX excretion in the IPRK

Table 4 contains renal excretion parameters for MTX in the IPRK. In vitro perfusate binding of MTX was approximately 89%. However, MTX binding was dramatically reduced in the presence of probenecid. As noted in the table, MTX excretion was significantly affected by co-administration of both probenecid and cimetidine. The lower clearance of MTX in the absence of interactant can be explained, in part, by lower GFR values in that study group. However, significant differences in XR were observed among study groups. Co-administration with probenecid resulted in increased MTX clearance, although XR value was considerably reduced. MTX clearance and XR were increased in the presence of cimetidine.

Discussion

VX-702 is a novel MAPK inhibitor under development for the treatment of RA. In this investigation, IPRK experiments were conducted to characterize the renal excretion of VX-702 across several doses. In addition, known inhibitors of specific renal transport systems were used to probe mechanisms of VX-702 excretion in the IPRK. The results were compared to a parallel series of experiments with MTX. Given the potential for combination therapy of VX-702 and MTX in RA patients, it is important to evaluate whether they share a common renal transport pathway that could potentially result in a toxic drug–drug interaction in the clinical setting.

The renal proximal tubule is a primary site of active secretion and reabsorption of various endogenous substrates and xenobiotics. A number of transport systems have been identified in the kidney that mediate drug transport at the basolateral (blood → kidney) and luminal (kidney → urine) membranes of the tubular cell (Figure 2). Two major drug transport systems in the

Table 1. Summary of IPRK viability parameters.

		VX-702 excretion studies					MTX excretion studies		
IPRK viability parameter	Control	VX-702 (100 ng/mL)	VX-702 (300 ng/mL)	VX-702 (600 ng/mL)	VX-702 and probenecid	VX-702 and cimetidine	MTX (500 ng/mL)	MTX and probenecid	MTX and cimetidine
Perfusion pressure (mmHg)	103 (2.31)	96.3* (6.82)	101 (3.66)	97.4* (5.05)	104 (2.57)	103 (1.50)	99.5* (4.86)	98.6* (3.78)	104 (6.18)
Perfusion flow rate (mL/min)	28.6 (4.72)	35.2* (10.0)	29.1 (7.62)	35.7* (6.32)	35.2* (11.9)	21.8* (4.19)	18.6* (3.96)	27.2 (6.41)	20.8* (9.64)
Urine flow rate (mL/min)	0.109 (0.025)	0.110 (0.038)	0.087* (0.024)	0.103 (0.029)	0.087* (0.023)	0.104 (0.025)	0.106 (0.043)	0.106 (0.043)	0.103 (0.033)
Urine pH	6.9 (0.16)	6.9 (0.12)	6.9 (0.13)	6.8 (0.14)	7.6* (0.55)	6.9 (0.10)	7.0 (0.11)	7.0 (0.10)	6.8 (0.10)
GFR (mL/min)	0.840 (0.368)	0.843 (0.318)	0.850 (0.251)	0.787 (0.304)	0.999 (0.342)	0.841 (0.303)	0.673 (0.230)	0.847 (0.357)	0.870 (0.251)
FR_{Glu}[a]	0.930 (0.06)	0.95* (0.03)	0.95* (0.04)	0.96* (0.02)	0.96* (0.02)	0.97* (0.01)	0.97* (0.02)	0.98* (0.01)	0.98* (0.01)
FR_{Na}[b]	0.90 (0.04)	0.91 (0.06)	0.94* (0.02)	0.92 (0.05)	0.94* (0.03)	0.90 (0.05)	0.91 (0.04)	0.93 (0.02)	0.92 (0.03)

Data are expressed as mean (SD) of data points representing IPRK urine collection periods. Perfusion experiments (n = 3 per group) consisted of 10 urine collection periods. The results are based on a total of 30 data points.

[a]Fractional reabsorption of glucose by the kidney.

[b]Fractional reabsorption of sodium by the kidney.

*Indicates statistically significant differences in parameters as compared to the control ($P < 0.05$).

Table 2. VX-702 renal excretion parameters in the IPRK: dose-linearity studies.

Renal excretion parameter	VX-702 (100 ng/mL)	VX-702 (300 ng/mL)	VX-702 (600 ng/mL)
GFR (mL/min)	0.85 (0.31)	0.86 (0.27)	0.78 (0.28)
Cl (mL/min)	0.12 (0.052)	0.099* (0.030)	0.096* (0.028)
XR[a]	0.22 (0.099)	0.17 (0.031)	0.19 (0.086)

Data are expressed as mean (SD) of data points representing IPRK urine collection periods. Perfusion experiments (n = 3 per group) consisted of 10 urine collection periods. The results are based on a total of 30 data points.

[a]Renal excretion ratio, calculated as $Cl/[f_u \times \text{GFR}]$. f_u represents fraction of drug unbound in perfusate.

*Denotes significant difference in parameter estimate compared with VX-702 100 ng/mL study group ($P < 0.05$).

Table 3. VX-702 renal excretion parameters in the IPRK: transport inhibition studies.

Renal excretion parameter	VX-702 (600 ng/mL)	VX-702 and probenecid	VX-702 and cimetidine
GFR (mL/min)	0.74 (0.31)	1.0* (0.35)	0.88*,** (0.28)
Cl (mL/min)	0.097 (0.028)	0.128* (0.037)	0.102** (0.024)
XR[a]	0.20 (0.0996)	0.19 (0.072)	0.18 (0.049)
Protein binding (%)	26.7 (7.26)	27.3 (11.4)	33.5 (11.4)

Data are expressed as mean (SD) of data points representing IPRK urine collection periods. Perfusion experiments (n = 3 per group) consisted of 10 urine collection periods. The results are based on a total of 30 data points.

[a]Renal excretion ratio, calculated as $Cl/[f_u \times \text{GFR}]$. f_u represents fraction of drug unbound in perfusate.

*Denotes significant difference in parameter estimate compared with VX-702 600 ng/mL study group ($P < 0.05$).

**Denotes significant difference in parameter estimate compared with VX-702 and probenecid study group ($P < 0.05$).

Table 4. MTX renal excretion parameters in the IPRK: transport inhibition studies.

Renal excretion parameter	MTX (500 ng/mL)	MTX and probenecid	MTX and cimetidine
GFR (mL/min)	0.68 (0.22)	0.88* (0.29)	0.89* (0.25)
Cl (mL/min)	0.069 (0.024)	0.193* (0.059)	0.116*,** (0.035)
XR[a]	0.93 (0.14)	0.23* (0.035)	1.2*,** (0.26)
Protein binding (%)	89.3 (0.71)	6.5* (6.0)	89.4 (0.74)

Data are expressed as mean (SD) of data points representing IPRK urine collection periods. Perfusion experiments (n = 3 per group) consisted of 10 urine collection periods. The results are based on a total of 30 data points.

[a]Renal excretion ratio, calculated as $Cl/[f_u \times \text{GFR}]$. f_u represents fraction of drug unbound in perfusate.

*Denotes significant difference in parameter estimate compared with MTX study group ($P < 0.05$).

**Denotes significant difference in parameter estimate compared with MTX and probenecid study group ($P < 0.05$).

kidney that have been identified and extensively characterized are the OAT and OCT systems[17,18].

VX-702 excretion displays linear excretion kinetics in the IPRK model. XR was less than 1 across all dosing groups, consistent with net reabsorption. To further clarify the mechanism(s) of renal VX-702 transport, the effect of known transporter inhibitors of OAT (probenecid) and OCT (cimetidine) pathways on VX-702 excretion were examined in the IPRK. Because concomitant administration of these transport inhibitors did not alter VX-702 excretion data (e.g., XR), it appears that VX-702 is not a substrate for renal OAT or OCT pathways.

The renal excretion of MTX has been previously studied in the IPRK[2,19]. MTX is highly protein bound. In this study, MTX was ~89% bound in perfusate. This estimate is consistent with a published data[19]. Co-administration of cimetidine had no effect on MTX binding. However, drug binding was significantly reduced in presence of probenecid (89% → 6%).

In this investigation, MTX renal excretion was evaluated at a clinically relevant concentration (500 ng/mL). The estimate of XR generated in IPRK experiments (0.93, Table 4) matches data reported by Statkevich et al. (XR = 0.93, MTX concentration 1 μg/mL). In that study, MTX excretion in the IPRK was nonlinear over a range of concentrations from 1 to 25 μg/mL, and clearance and XR were significantly increased with dose[2]. MTX demonstrated net reabsorption at the lowest concentration and net secretion at the other concentrations studied (based on XR data). A subsequent IPRK study reported an MTX XR of approximately 1.5 at a concentration of 25 μg/mL, consistent with the Statkevich study[19]. Collectively, these IPRK data indicate that MTX excretion involves a combination of mechanisms (filtration, secretion, and reabsorption). Furthermore, it appears that several transport systems likely mediate the renal excretion of MTX. This is supported by transport inhibition studies carried out in this study.

Co-administration of probenecid significantly reduced MTX binding (89% → 6%). As a result, MTX clearance would be expected to increase as a result of increased filtration. In these IPRK experiments, MTX clearance was significantly higher in the presence of probenecid. However, XR ratio was significantly lower, consistent with inhibition of MTX secretion by the kidney. Overall, these results suggest that MTX is a substrate for OAT system(s) in the kidney. In cases where renal tubular transport is inhibited without affecting protein binding, reduced MTX clearance would be expected. This pattern has been demonstrated when MTX is co-perfused with NSAIDs (e.g., indomethacin and ketoprofen). NSAIDs significantly reduced MTX clearance with no effect on perfusate binding[2,19]. The

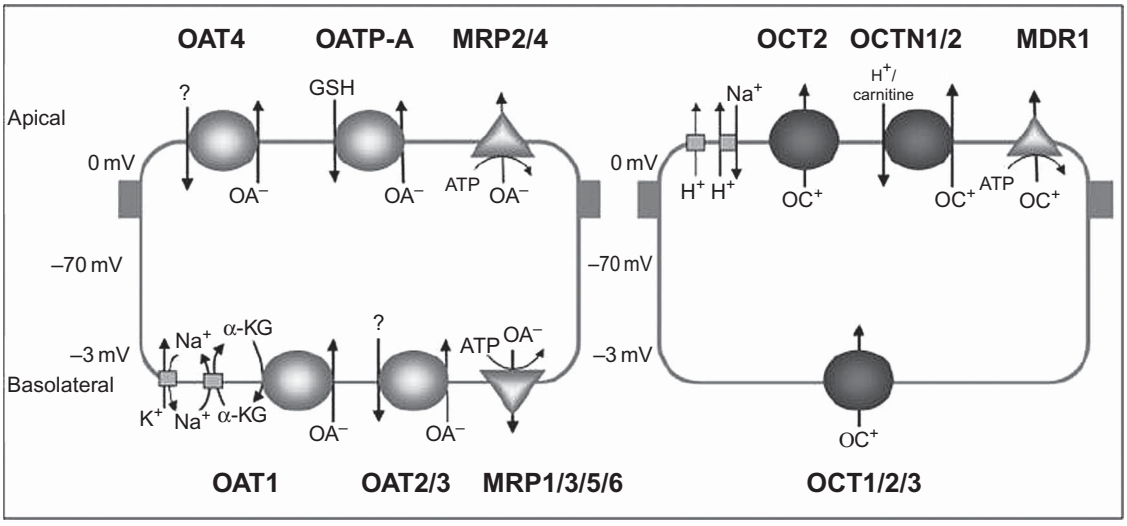

Figure 2. Organic anion and organic cation transporters located on apical and basolateral membrane of the kidney cell. Reproduced with permission from annual reviews: Lee and Kim[17], page 146, Figure 1.

MTX–NSAID interaction has been associated with toxic manifestations, including several deaths in patients treated with these medications[4,5].

Study results of this investigation also suggest involvement of OCT on MTX excretion in the IPRK. Coadministration of cimetidine resulted in higher MTX XR (0.93 → 1.2). This could be explained by reduced luminal uptake (i.e., reabsorption) of MTX in the presence of cimetidine, resulting in net secretion of MTX. As discussed above, MTX has demonstrated dose-dependent increases in clearance in the IPRK. This would suggest active reabsorption of drug in the kidney, a finding supported by this interaction study with cimetidine.

Conclusions

VX-702 exhibited dose-linear excretion in the IPRK, and clearance data were consistent with net reabsorption by the kidney. Transport inhibition studies indicate that VX-702 is not a substrate for renal OATs and OCTs. Coadministration of probenecid and cimetidine significantly altered the renal excretion of MTX in the IPRK, pointing to a role of these transport pathways on MTX excretion. Overall, the results of this research suggest that a renal drug–drug interaction between VX-702 and MTX is unlikely.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this paper.

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