Inhibition of OCT2, MATE1 and MATE2-K as a possible mechanism of drug interaction between pazopanib and cisplatin


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We hypothesized that pazopanib is an inhibitor of cisplatin renal transporters OCT2, MATE1 and MATE2-K based on previous studies demonstrating an interaction between tyrosine kinase inhibitors and these transporters. Because several combinations of targeted therapies and cytotoxics are currently in development for cancer treatment, such an interaction is worth investigating. Experiments on HEK293 cells stably transfected to express OCT2, MATE1, MATE2-K or an empty vector (EV) were conducted. The inhibitory effect of pazopanib on these transporters was measured using the uptake of fluorescent substrate ASP+ and cisplatin in different cell lines. The effect of pazopanib on cisplatin-induced cytotoxicity was also evaluated. A decrease of ASP+ uptake was observed in OCT2-HEK, MATE1-HEK and MATE2K-HEK cell lines after addition of pazopanib at increasing concentrations. Pazopanib inhibited cisplatin specific uptake in OCT2-HEK, MATE1-HEK and MATE2K-HEK lines. Cytotoxicity experiments showed that co-incubation of cisplatin with pazopanib multiplied up to 2.7, 2.4 and 1.6 times the EC50 values of cisplatin in OCT2-HEK, MATE1-HEK and MATE2K-HEK cell lines respectively, reaching about the same values as in EV-HEK cells. To conclude, pazopanib inhibits OCT2, MATE1 and MATE2-K, which are involved in cisplatin secretion into urine. The combination of these two drugs may lead to an interaction and increase the cisplatin-induced systemic toxicity. Given the wide variability of plasma pazopanib concentrations observed in vivo, the interaction may occur in a clinical setting, particularly in overexposed patients. The existence of a drug–drug interaction should be investigated when pazopanib is associated with a substrate of these transporters.

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1. Introduction

Angiogenesis inhibitors represent a promising approach in the treatment of many cancers as their combination with chemotherapy may potentiate the antitumor activity [1]. Pazopanib is a multi-targeted tyrosine kinase inhibitor with anti-angiogenic activity targeting the vascular endothelial growth factor receptor (VEGFR), platelet-derived growth factor receptor (PDGFR), and c-KIT. Pazopanib given orally at 800 mg once daily is approved for the treatment of advanced renal cell carcinoma and some sarcomas [2]. Pazopanib is known to be subject to drug–drug interactions (DDI) as a substrate of CYP3A4, ABCB1 (known as P-glycoprotein) and ABCG2 efflux proteins [3,4]. Furthermore, it inhibits CYP1A2, 3A4, 2B6, 2C8, 2C9, 2C19, and 2E1 and human organic anion transporter (OATP1B1) [5,6]. So far, there is no data concerning interactions between pazopanib and the renal transporters OCT2, MATE1 and MATE2-K which could result in a DDI with cisplatin, a substrate of these carriers. Cisplatin is indicated for the treatment of many solid tumours, and is usually used in combination with other chemotherapies. The balance of uptake and efflux by OCT and MATE could determine the renal accumulation and the nephrotoxicity of cisplatin [7]. OCT2 is expressed at the basolateral membrane of the renal proximal tubular cells and mediates uptake of its substrates. MATE1 and MATE2-K are on the brush-border membrane and lead
to excretion of the substrates. Cisplatin is now considered as a substrate of both MATE1 and MATE2-K [8] although an earlier study showed that cisplatin is preferentially transported by MATE1 [9].

We hypothesized that pazopanib could inhibit OCT2 and/or MATE1 and/or MATE2-K based on previous studies demonstrating an interaction between other tyrosine kinase inhibitors (TKI) and these transporters. Indeed, Tanhara et al. [10] showed that the concomitant administration of imatinib with cisplatin prevents cisplatin-induced nephrotoxicity, mainly due to the inhibition of OCT2-mediated renal accumulation of cisplatin, using transporter-expressing cells and rats. OCT2 inhibition by imatinib led to a decrease of cisplatin clearance and an increase of the plasma cisplatin AUC (area under the curve) in rats. Recently, Shen et al. [11] showed that vandetanib inhibits OCT2, MATE1 and MATE2-K transporters in vitro and can also reduce cisplatin-induced cytotoxicity of MATE1- and MATE2K-transfected HEK293 cells. A larger screening of eight TKIs [12] demonstrated that imatinib, dasatinib, nilotinib, gefitinib, erlotinib, and sunitinib exert selectively potent inhibitory effects on MATE1, OCT3, MATE2-K, and OCT1.

The aim of this study was to evaluate in vitro whether pazopanib can inhibit the transporters OCT2, MATE1, and MATE2-K and estimate the impact of this inhibition on the transport of cisplatin. Since the phase I clinical trial was conducted to evaluate the maximum tolerated dose of pazopanib in combination with cisplatin showed a global poor tolerance (PACIFIK study, EudraCT n° 2009-016781-86), the question of the existence of OCT2, MATE1 and MATE2-K inhibition by pazopanib is clinically relevant.

2. Materials and methods

2.1. Cell lines

Three cell lines were obtained to stably express respectively human OCT2 or MATE1 or MATE2-K transporters. A fourth line transfected with an empty vector (EV) was used as a negative control. These four lines (OCT2-HEK, MATE1-HEK, MATE2K-HEK, EV-HEK) were kindly provided by Dr. KM Giacomini (Department of Bioengineering and Therapeutic Sciences, University of California San Francisco, San Francisco) who validated these cell models in previous studies [12–14]. They were established by stably transfecting pcDNA/FRT vector (Invitrogen, Carlsbad, CA) containing the gene of interest into HEK293 (Human Embryonic Kidney) Flp-in cells using Lipofectamine 2000 (Invitrogen, Carlsbad, CA) according to the manufacturer instructions. Real-time PCR results showed significant overexpression of OCT2, MATE1 and MATE2-K mRNA in transfected cells compared with EV cells (data not shown).

2.2. Cell culture

Stable transfected HEK293 cells were cultured in Dulbecco’s modified Eagle’s medium (Pan Biotech, Aidenbach, Germany) containing 10% fetal calf serum (Pan Biotech, Aidenbach, Germany), 1% L-glutamine (Sigma, St. Louis, MO), 1% penicillin/streptomycin (Pan Biotech, Aidenbach, DE), and 200 µg/ml hygromycin (Sigma, St. Louis, MO) in an atmosphere with 5% CO2 and 95% air at 37°C.

2.3. ASP+ uptake assay

ASP+ (4-[4-(dimethylamino)styryl]-N-methylpyridinium iodide) is a known substrate of OCTs [15] and MATE [8,14] and was purchased from Life Technologies (Carlsbad, CA). Cells were seeded in black poly-ω-lysine-coated 96-well plates (Greiner, GmbH Pleidelsheim, Germany) at 100,000 cells per well and grown for 48 h. Cells lines were washed twice with Hank’s Balanced Salt Solution (HBSS, Pan Biotech). In order to acidify the cytosol and reverse the transport direction of MATE transporters, MATE1-HEK and MATE2K-HEK cells were pre-incubated for 30 min in a 30 mM NH4Cl solution in HBSS at pH 6.5 while OCT2-HEK and EV-HEK were pre-incubated in HBSS. The pre-incubation medium was then removed and uptake was initiated by addition of ASP+ at concentrations ranging from 0 to 200 µM. After incubating for 5 min at 37°C, reaction was stopped by aspirating the substrate and washing the cells twice with ice-cold HBSS containing 500 µM cimetidine (Sigma, St Louis, MO) to stop the reaction. Cimetidine is a well-known inhibitor of OCT2, MATE1 and MATE2-K transporters [13,16]. Fluorescence was measured before and after addition of ASP+ with a plate reader Infinite F200 pro (Tecan, Lyon, France). Cells were lysed with 100 µl of RIPA buffer (Tris 50 M, Triton 1%, SDS 0.1%, MgCl2 5 mM, NaCl 150 mM, sodium deoxycholate 0.5%) incubated for one hour at 4°C. The protein concentration of the solubilized cells was determined using a bichinchoninic acid (BCA) Protein Assay Kit (Sigma, St. Louis, MO). Km was calculated with GraphPad Prism (GraphPad Software version 5, Inc., San Diego, CA) by fitting the experimental data with the Michaelis-Menten equation (Y = Vmax × X/(Km + X)) where Y is the ASP+ uptake velocity of and X the ASP+ concentration.

2.4. Cisplatin uptake experiment

Cells were seeded in poly-ω-lysine-coated 24-well plates (Greiner) at 450,000 cells per well and grown for 48 h. Cells of all four lines were washed twice with Hank’s Balanced Salt Solution (HBSS, Pan Biotech). After the 30 min pre-incubation in the NH4Cl solution (30 mM, pH 6.5) for MATE1-HEK and MATE2K-HEK cells and HBSS for OCT2-HEK and EV-HEK, uptake was initiated by addition of cisplatin at 20 µM or 1000 µM. The reaction was stopped after 2 h of incubation by aspirating the substrate and washing the cells twice with ice-cold HBSS containing 500 µM cimetidine. Cells were lysed with 300 µl of RIPA buffer incubated for one hour at 4°C. The protein concentration of the solubilized cells was determined using a Bio-Rad Protein Assay Kit. Concentrations of cisplatin were measured by atomic absorption spectrometry PinAAcie900Z (PerkinElmer® on this lyase by a method routinely used in the laboratory [17]).

2.5. Assessment of ASP+ and cisplatin uptake inhibition

ASP+ uptake experiment was repeated by incubating the cells 1 h at 37°C with pazopanib (GSK, London, UK) at concentrations ranging from 0 to 20 µM before adding the fluorescent substrate ASP+ at 2.5 µM. The pazopanib stock solution was prepared in DMF (Dimethyformamide, Sigma St. Louis, MO) at 4 g/L and then diluted in HBSS without exceeding a final concentration of 0.5% of DMF. In order to take into account a putative effect of DMF on fluorescence and cell viability at this concentration, 0.5% DMF was also added to the blank. For each cell line, the IC50 (concentration of pazopanib that gives a response half way between the curve’s bottom and top) was calculated using the Prism software (GraphPad Software version 5, Inc., San Diego, CA) by fitting the experimental data with a built-in log(inhibitor) vs. response equation implemented in Prism: Y = 100/(1 + 10(logIC50-X) × HillSlope) where Y is the percentage of substrate quantity normalized to the control condition (no pazopanib, set to 100%), X is the log concentration of pazopanib, HillSlope describes the steepness of the curve and is determined by the software.

Cisplatin uptake experiment was repeated by adding pazopanib at concentrations ranging from 0 to 20 µM simultaneously to cisplatin at 20 µM or 1000 µM for 2 h.
2.6. Measurement of cytotoxicity

Cells lines were seeded in poly-d-lysine-coated 96-well plates (Greiner®) at 5000 cells per well and grown for 24 h. Cells were washed twice with HBSS. After a pre-incubation for 30 min in a 30 mM NH₄Cl solution in HBSS at pH 6.5 of MATE1-HEK and MATE2K-HEK cells or in HBSS for OCT2-HEK and EV-HEK, cisplatin was added in a range of 0–1000 μM with or without pazopanib. Cells treated with pazopanib were pre-incubated for one hour before addition of cisplatin with pazopanib 1 μM or 5 μM. After two hours of incubation of cisplatin with or without pazopanib, incubation solution was aspirated and culture medium was added. After 72 h, cells viability was measured using the WST-1 colorimetric assay (Roche Applied Science Mannheim Germany). Briefly, WST-1 solution was added to the cell in each well to obtain a final dilution at 1/10. After 2 h of incubation at 37 °C in an atmosphere with 5% CO₂ and 95% air, absorbance was measured at 450 nm with a plate reader Infinite F200 pro. The concentration of cisplatin that decreases the cell viability by 50% (EC50) was calculated with GraphPad Software version 5 using a built-in log(inhibitor) vs. response equation implemented in Prism:

\[ Y = \frac{100}{1 + 10^{(\text{LogEC50}-X) \times \text{HillSlope}}} \]

where Y is the percentage of viable cells normalized to the control condition (no cisplatin, set to 100%), X is the log concentration of cisplatin, HillSlope describes the steepness of the curve and is determined by the software.

2.7. Statistical analysis

All data are reported as mean ± SEM (standard error of the mean). Means were calculated from a minimum of three distinct experiments, with duplicates within any single experiment being averaged to obtain a single value for that experimental series. Cisplatin uptake in OCT2-HEK cells, MATE1-HEK cells and MATE2K-HEK cells were compared to EV-HEK cells using Mann-Whitney tests. The effect of increasing concentrations of pazopanib on cisplatin accumulation was tested by comparing the uptake at each pazopanib concentration level to the control (100%) with Mann-Whitney tests. For cytotoxicity experiments, intrinsic pazopanib cytotoxicity was evaluated by comparing the cell viability in the blank (no cisplatin) with 0, 1 or 5 μM of pazopanib with a Kruskall-Wallis test. The difference between the cisplatin EC50 values in EV-HEK treated at 0, 1 or 5 μM of pazopanib concentration was also tested using a Kruskall-Wallis test. In order to evaluate the effect of pazopanib on cisplatin-induced cytotoxicity, the resistance factor (RF) values were calculated for each condition in each experiment as previously proposed by Shen et al. [11]. The RF was defined as the ratio of the EC50 value in EV-HEK cells to that in the corresponding transfected cell line and is a means of assessing the sensitivity of the transfected cell line compared to the control cell line (HEK-EV). A RF was calculated with the EC50 obtained at each individual experiment and were compared to 1 using Student t-tests. All statistical tests are performed at the 5% significance level.

All statistical analyses were performed using Stata 13 (StataCorp. 2013. Stata Statistical Software: Release 13. College Station, TX: StataCorp LP).

3. Results

3.1. ASP+ uptake assay

The uptake of ASP+ by OCT2-HEK cells, MATE1-HEK cells, and MATE2K-HEK cells was higher than in EV-HEK cells (Fig. 1A and 1B), demonstrating the functional activity of each transporter. Km were calculated respectively at 13.9 ± 3.3 μM; 3.2 ± 1.8 μM; 5.4 ± 1.7 μM for OCT2-HEK, MATE2K-HEK, and MATE1-HEK. An ASP+ concentration of 2.5 μM (<<Km) during 5 min was chosen for the following experiments as it was in the linear range of the transport kinetics.

3.2. Cisplatin uptake experiment

Cisplatin uptake experiments were carried out with cisplatin at 20 μM and 1000 μM. The measured intracellular concentration of cisplatin was 61, 53, 54 and 52 times higher in the experiments performed at 1000 μM compared to 20 μM, for OCT2-HEK, MATE1-HEK, MATE2K-HEK and EV-HEK cell lines respectively. Cisplatin uptake was evaluated by dividing the cisplatin concentrations measured in the cell lysate by the protein concentration measured with BCA in each transfected cell line. The results are depicted in the Supplementary Fig. S1A and B in the online version at DOI: 10.1016/j.phrs.2016.05.012. Cisplatin uptake was 1.6, 1.9 and 1.8 fold higher in OCT2-HEK cells, MATE1-HEK cells and MATE2K-HEK respectively than in EV cells after incubation of cisplatin 20 μM for 2 h. These ratios were at 1.8, 2.1 and 2 after incubation of cisplatin 1000 μM.

![Fig. 1](image-url) Concentration dependence of ASP+ uptake into transfected HEK cell lines. A: Range from 0 to 200 μM of ASP+. B: Focus on range from 0 to 10 μM of ASP+.
for 2 h. The differences between transporter- and EV-transfected cell lines were significant with \( p = 0.011, p = 0.006 \) and \( p = 0.002 \) for the experiment with cisplatin 20 \( \mu \text{M} \) and \( p = 0.002, p = 0.004 \) and \( p = 0.004 \) for the experiment with cisplatin 1000 \( \mu \text{M} \) in OCT2-HEK cells, MATE1-HEK cells and MATE2K-HEK cells respectively.

3.3. Effect of pazopanib on ASP+ uptake

Pazopanib was evaluated as an inhibitor of OCT2, MATE1 and MATE2-K transporters using ASP+ substrate. A decrease of ASP+ specific uptake was observed in OCT2-HEK cells, MATE1-HEK cells and MATE2K-HEK cells after addition of pazopanib at increasing concentration from 0 to 20 \( \mu \text{M} \) (Fig. 2). ASP+ specific uptake was evaluated as the ratio of fluorescence divided by protein concentrations in OCT2-HEK, MATE1-HEK and MATE2K-HEK cells to which the corresponding ratio obtained in EV-HEK was subtracted. As a result, the decrease of ASP+ specific uptake observed after addition of pazopanib can be attributed to an inhibition of OCT2, MATE1 and MATE2-K transporters by pazopanib. The IC50 and Ki values for this experiment are shown in Table 1.

3.4. Effect of pazopanib on cisplatin uptake

Regarding the transport of cisplatin, the addition of increasing concentrations of pazopanib resulted in a decrease of cisplatin specific uptake in the three cell lines after incubation of cisplatin 20 \( \mu \text{M} \) (Fig. 3A) or 1000 \( \mu \text{M} \) (Fig. 3B) for 2 h. Cisplatin specific uptake was evaluated as the ratio of intracellular concentrations of cisplatin divided by protein concentrations in OCT2-HEK cells, MATE1-HEK cells and MATE2K-HEK cells to which the corresponding ratio obtained in EV-HEK was subtracted. As a result, the decrease of cisplatin specific uptake observed after addition of pazopanib can be attributed to an inhibition of OCT2, MATE1 and MATE2-K transporters by pazopanib.

3.5. Effect of pazopanib on cisplatin-induced cytotoxicity

To confirm these results and evaluate the effect of pazopanib on cisplatin-induced cytotoxicity, cell viability was measured after incubation of cisplatin with or without pazopanib (Fig. 4). Pazopanib [1 \( \mu \text{M} \)] or [5 \( \mu \text{M} \)] itself did not exhibit any cytotoxicity under the same test conditions (Supplementary Fig. S2 in the online version at DOI: 10.1016/j.phrs.2016.05.012). In the absence of pazopanib, the EC50 values of cisplatin in OCT2-HEK, MATE1-HEK and MATE2K-HEK cells after 72 h of drug exposure were all lower than those in EV cells confirming that a higher amount of cisplatin had entered the cell via the transporters. The resistance factors obtained for OCT2-HEK or MATE2K-HEK indicate that the absence of OCT2 or MATE2-K confers to EV cells a 3-fold resistance compared to transporter-transfected cell lines. Co-incubation of cisplatin with pazopanib (1 or 5 \( \mu \text{M} \)) resulted in an increase of EC50 values up to 2.7 and 2.4 times compared to the EC50 values of cisplatin alone in OCT2-HEK cells, MATE1-HEK cells respectively, reaching approximately the same values as the EC50 values observed in EV 19.5 and 17.6 \( \mu \text{M} \) for OCT2-HEK and MATE1-HEK vs. 18.3 \( \mu \text{M} \) for EV-HEK. In MATE2K-HEK cells, co-incubation with pazopanib had a lower effect than in OCT2-HEK cells and MATE1-HEK cells but EC50 values increased by up to 1.6. No significant difference was observed between the EC50 values in EV-HEK treated at 0, 1 or 5 \( \mu \text{M} \) of pazopanib concentration (\( p = 0.82 \)), confirming that pazopanib exerts its effect through the transporters. The resistance factor values (RF) and p-values are shown in Table 2. These results show that the RFs in absence of pazopanib are significantly different from 1 (RF value for the control EV cell line) for the 3 cell lines. In each transfected cell line, the addition of pazopanib at 1 \( \mu \text{M} \) and 5 \( \mu \text{M} \) induces a decrease of RF which tends towards 1. In the condition with cisplatin + pazopanib 5 \( \mu \text{M} \), the RF for the 3 cell lines are not significantly different from 1. Therefore, at this concentration, the inhibition of OCT2, MATE1

![Fig. 2.](image-url) Effect of pazopanib on the ASP+ uptake into transporter-transfected HEK cell lines. The specific uptake of ASP+ was calculated by subtracting the uptake by EV-HEK cells from the uptake by OCT2-HEK cells, MATE1-HEK cells and MATE2K-HEK cells. Data are presented as mean ± SEM (n = 4 distinct experiments with each experimental condition repeated twice).

![Fig. 3.](image-url) Effect of pazopanib on the cisplatin uptake into transporter-transfected HEK cell lines. Cells were incubated with cisplatin 20 \( \mu \text{M} \) (A) or 1000 \( \mu \text{M} \) (B) during 2 h in the presence of pazopanib at the designated concentrations. The specific uptake of cisplatin was calculated by subtracting the cisplatin accumulation in EV-HEK cells from the cisplatin accumulation in OCT2-HEK cells, MATE1-HEK cells and MATE2K-HEK cells. For each cell line, the condition without pazopanib was set at 100% as the control value. Data are presented as mean ± SEM (n = 3 distinct experiments with each experimental condition repeated twice).

*: \( p < 0.05 \) (compared to control).
An increased uptake of ASP+ and cisplatin in OCT2-, MATE1- and MATE2K-HEK cells compared to the negative control line EV was observed and validated these cell lines as adequate models to further study the inhibitory effect of pazopanib.

In the cisplatin experiments, the ratio of transfected cell lines data on EV data varies between 1.6 and 2.1, while in the experiments with the ASP+ substrate, this ratio was between 2 and 13 times higher than in the EV line. This difference can probably be explained by a greater passive diffusion of cisplatin across cell membranes compared to ASP+. Indeed, whatever the experiment, the intracellular concentration of cisplatin obtained with the EV line was stable showing that pazopanib has no effect on this passive diffusion.

The first step was to study the effect of pazopanib on OCT2, MATE1 and MATE2-K using ASP+ as a probe substrate. Our results indicate that pazopanib is a potent inhibitor of these three transporters with IC50 values in the micromolar range as shown in Table 1.

Specifically, we showed that pazopanib significantly inhibits cisplatin uptake by OCT2, MATE1 and MATE2-K transporters. These results concur with previous studies [10–12] that showed that several TKI are inhibitors of these transporters. In our experiments, it was not possible to reach the complete inhibition of ASP+ or cisplatin uptake at the highest concentration tested (20 μM). The poor solubility of pazopanib did not allow the use of higher concentrations than 20 μM.

Cytotoxicity experiments were performed as another tool to evaluate the amount of cisplatin accumulating inside the cell. They confirmed that pazopanib inhibited the uptake of cisplatin via these three transporters which resulted in a decreased cisplatin-cytotoxicity in OCT2-, MATE1- and MATE2K-HEK cells. The EC50 increase was higher in OCT2 and MATE1 cells as compared to MATE2-K cells. In the latter, pazopanib at 5 μM seemed not to be potent enough to completely inhibit cisplatin uptake by MATE2-K. However, even though the EC50 obtained with pazopanib at 5 μM did not reach the EC50 of the control EV cell line, the RF in this condition was not statistically different from 1 which means that the part of cytotoxicity due to cisplatin uptake by MATE2-K is negligible.

To put these results in the clinical pharmacologic context, it should be noted that in our experiments with MATEs, the intracellular medium was acidified to invert the proton exchange as described in previous studies [10,12,14] and therefore, cisplatin entered the cells via MATE1 and MATE2-K whereas pharmacologically, cisplatin is secreted into urine by these transporters. Consequently, OCT2 inhibition by pazopanib may lead to an increase of systemic adverse effects of cisplatin such as hematoxicity but could be nephroprotective [10,18]. On the contrary, MATE1 and MATE2-K inhibition may increase the nephrotoxicity of cisplatin by increasing its tubular intracellular concentrations [19]. Therefore the effect of this triple inhibition on nephrotoxicity is difficult to predict. In line with this issue, Sproawl et al. [20] studied the impact of conjunctive therapy of cisplatin with cimetidine in 19 patients. Indeed, as an OCT2 inhibitor, cimetidine has previously shown a nephroprotective effect on cisplatin-induced renal toxicity in vitro [18]. The clinical study showed that the use of cimetidine had no influence on unbound cisplatin plasma clearance. Therefore, it is possible that the inhibition of these transporters leads to

Table 1

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<thead>
<tr>
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<th>Pazopanib IC50 (μM)</th>
<th>% of maximum inhibition</th>
<th>Ki (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>OCT2</td>
<td>3.5 (1.2)</td>
<td>89.4</td>
<td>3.0</td>
</tr>
<tr>
<td>MATE1</td>
<td>3.1 (1.4)</td>
<td>64.9</td>
<td>1.7</td>
</tr>
<tr>
<td>MATE2-K</td>
<td>4.8 (1.3)</td>
<td>64.1</td>
<td>3.3</td>
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</table>

and MATE2-K results in a cisplatin-induced cytotoxicity similar to that of the EV-HEK control cell line.

4. Discussion and conclusions

This study is the first to demonstrate that pazopanib is an inhibitor of OCT2, MATE1 and MATE2-K renal transporters. This inhibition results in a decrease of cisplatin uptake by these transporters and therefore attenuates cisplatin-induced cytotoxicity in vitro.
cellular effects that would not be translated into modifications of the drug disposition in the whole body. In the phase I study combining cisplatin and pazopanib, the PK study [21] did not reveal a clear impact of pazopanib on cisplatin clearance, however this latter significantly decreases during treatment (cycle 2 vs. cycle 1) and it cannot be excluded that pazopanib given daily for more than 14 days, participated to the observed decrease of cisplatin clearance. Imbs et al. reported a global poor tolerance of the combination [21].

A limitation of our work is the use of 3 independent monotransfected cell lines that do not allow us to quantify the global effect of pazopanib on cisplatin elimination at the tubular cell level expressing many transporters. However, our goal was to provide a first evaluation of the relevance to study a clinical DDI between pazopanib and cisplatin, via an inhibitory effect of pazopanib on OCT2, MATE1 and MATE-2K.

To investigate the clinical relevance of this study, we compared the concentrations of drugs used in our experiments to those found in human plasma. In the PACIFIK study [21], the maximum plasma ultrafiltrable cisplatin concentrations representing the unbound fraction were about 20 μM. All our experiments were conducted at 1000 μM and repeated at 20 μM corresponding to this observed unbound cisplatin concentration. Regarding pazopanib, the mean total C\textsubscript{max} was 84 μM and displayed a high variability (range: 10–190 μM). Given the extensive binding to plasma protein, i.e., >99.5% [22], the unbound C\textsubscript{max} was ranging between 10 nM and 190 nM. In our experiments, the concentration used for pazopanib was comprised between the unbound and total plasma fraction (range of 312 nM to 20 μM). As a result, our experimental conditions seem to be representative of the clinical situation. Besides the unbound concentration of pazopanib, we can hypothesize that the relative affinity of pazopanib for OCT2 over plasma proteins could increase the amount of pazopanib that will bind to OCT2 and may increase the risk for the DDI to occur.

Concerning the MATEs, one may wonder whether the relevant drug concentration to consider could be the intracellular pazopanib concentration within the tubular cell rather than the plasma unbound concentration. Given the high lipophilicity (logP = 3.65), the good permeability of pazopanib [22,23] and the ability to enter the cell via passive diffusion (data not shown), it can be inferred that it would inhibit MATE transporters but the extent of this inhibition in vivo is difficult to predict. Overall, although the DDI between pazopanib and cisplatin may not occur in all patients, the extremely important variability in pazopanib concentrations [23] may cause a DDI in overexposed patients.

Moreover, Wittwer et al. [14] showed that the IC\textsubscript{50} is dependent on the substrate so the existence of a DDI between pazopanib and other substrates of OCT2, MATE1 and MATE-2K (such as metformin) should be investigated.

According to these results, if the clinical benefit of the pazopanib- cisplatin association is confirmed and further used as a chemotherapy protocol, the nephrotoxicity should be carefully monitored in vivo given the inhibitory properties of pazopanib observed in vitro.

5. Conclusion

The present study revealed pazopanib as an inhibitor of OCT2, MATE1 and MATE2-K and highlights the need of such DDI studies before considering an association with other treatments which are substrates of OCT2 and/or MATE1 and/or MATE2-K.

Conflicts of interest statement

None
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