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Interaction of human multidrug and toxin extrusion 1 (MATE1) transporter with antineoplastic agents

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Abstract

Background: The transport of endogenous and exogenous organic cations across the plasma membrane of cells is mediated by multispecific organic cation transporters (OCTs), and the multidrug and toxin extrusion (MATE) transporters. MATE belongs to the SLC47 transporter family consisting of only two members, MATE1 and MATE2-K. MATE2-K is exclusively expressed in the kidney at the apical membrane of proximal tubular epithelial cells. MATE1 is highly expressed in the kidney, liver, skeletal muscle and also in adrenal glands, testes and heart. MATE1 exchanges organic cations against protons both in influx as well as in efflux modes.

Methods: Here, we examined the interaction of 25 antineoplastic agents with human MATE1. We generated stably transfected MATE1-HEK293 cells and determined the inhibition of MATE1-mediated [^{3}H]1-methyl-4-phenylpyridinium (MPP) uptake by the antineoplastic agents.

Results: We found a significant inhibition of MATE1-mediated MPP uptake by several antineoplastic agents and pH dependent IC_{50} values for mitoxantrone (7.8 μM at pH 7.4 and 0.6 μM at pH 8.5) as well as for irinotecan (4.4 μM at pH 7.4 and 1.1 μM at pH 8.5), respectively.

Conclusions: We suggest that hMATE1 could play a role in chemosensitivity of tumor cells. In addition, hepatic and renal MATE1 could potentially be involved in drug-drug-interactions as well as in drug metabolism and excretion during chemotherapy.

Keywords: antineoplastic agents; MATE1; SLC-transporter.

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Introduction

Resistance of tumor cells remains a challenging problem in chemotherapy. One out of several reasons for chemoresistance is the efficient export of antineoplastic agents from tumor cells by ATP-binding-cassette (ABC) transporters, so that the tumor cells are not efficiently affected by these drugs. Another problem of chemotherapy are drug-drug interactions and adverse effects. Drugs can interact with transporters and prevent physiological substrates being handled by these proteins. This can lead to a dysfunction or imbalance of essential transport processes of endogenous substances or drugs and, consequently, toxicity at the level of the organs expressing these transporters or for the whole organism, particularly when major excretory routes in liver and kidneys are involved.

In the last decade, intensive research was undertaken on the functional understanding of transport proteins in the combat against drug-drug interactions. A few years ago, a promising transport protein was identified that belongs to the solute carrier 47 family (SLC47). This protein was named multidrug and toxin extrusion 1 transporter (1). Human MATE1 (hMATE1) was described to mediate the transport of various endogenous substances, drugs and toxins (2, 3).

The MATE transporters were described in 1998 when the first members, the transport proteins NorM from *Vibrio parahaemolyticus* and YdhE from *Escherichia coli*, were established (4). In 1999, these two transporters were identified as members of a new transporter family, multidrug and toxin extrusion (MATE) (5). The human MATE1 consists of 570 amino acids. Otsuka et al. predicted 12 putative transmembrane domains (1). Terada and Inui reported 13 putative-transmembrane domains, with an intracellular N-terminus and extracellular C-terminus by using MATE1 amino acid sequences from various species and several transmembrane domain predicting programs (6).

The hMATE1 exchanges its substrates against protons. Consequently substrate uptake is driven by an in-to-out proton gradient with a maximum uptake activity at an extracellular pH of 8.5 (1). Substances transported by hMATE1 include organic cations, such as metformin, tetraethylammonium (TEA) and 1-methyl-4-phenylpyridinium (MPP), a few anions, such as estrone-3-sulfate and also zwitterionic molecules and drugs including the antibiotics cephalexin, cephradine and fluoroquinolones (2, 3). However, there is limited data on the ability to transport antineoplastic agents. Two platinum agents and mitoxantrone have been described as substrates or inhibitors for hMATE1 (7, 8).

The aim of this study was to elucidate the interaction of hMATE1 stably expressed in HEK293 cells with 25 antineoplastic agents.

Materials and methods

Reagents

Tritium labeled methyl-4-phenylpyridinium iodide ([^{3}H]-MPP) was purchased from ARC (St. Louis, MO, USA), antineoplastic agents were purchased from Sigma Aldrich (Taufkirchen, Germany) and the pharmacy department of the University Medicine of Göttingen (UMG), Germany. Further chemicals were obtained from Applichem (Darmstadt, Germany), Merck (Darmstadt, Germany) and Sigma Aldrich, at analytical grade. The transfection reagent lipofectamin was purchased from Invitrogen (Carlsbad, CA, USA), Dulbecco's modified eagle medium, high glucose (DMEM-HG) from Biochrom (Berlin, Germany), fetal calf serum (FCS) and trypsin from Gibco (Eggenstein, Germany) and penicillin/streptomycin and hygromycin from PAA (Pasching, Germany).

pDONR221, pEF5/FRT/V5-DEST and pOG44 plasmids, One Shot OmniMAX2 T1 Phage-Resistant Cells and Flp-In T-REx 293 cells were purchased from Invitrogen. The pCMV-SPORT6/hMATE1 plasmid in *E. coli* cells was purchased from Imagenes (Berlin, Germany).

Cell culture

Flp-In T-REx 293 cells expressing hMATE1 and non-expressing control cells were grown in a humidified atmosphere of 95% air and 5% CO_2 in DMEM-HG-medium supplemented with 10% (v/v) FCS and 1% (v/v) penicillin/streptomycin.

Plasmid isolation

Escherichia coli cells with the pCMV-SPORT6/hMATE1 plasmid were replicated in LB medium at pH 7.0 containing 10 g sodium chloride, 10 g trypton and 5 g yeast extract in 1 L double distilled water. The plasmid was isolated with the Compact Prep Plasmid Midi Kit (Qiagen, Hilden, Germany) according to the manufacturers' protocol.

Generation of a hMATE1-expression vector

Gateway Clonase system was used following the instructions by Invitrogen to clone the *hMATE1* gene into an expression vector. Briefly, at first the *hMATE1* gene was cloned from the pCMV-sport6/hMATE1 plasmid into the Donr221 plasmid generating a hMATE1 entry vector. Secondly, the *hMATE1* gene was cloned into the pEF5/FRT/V5-DEST vector generating a hMATE1 expression vector.

Cloned vectors were transformed into One-Shot-OmniMAX-2-T1-Phage-Resistant cells by heat shock following the protocol by Invitrogen. To replicate the transformed cells, 20 µL of the cloned products were isolated on agar plates [LB medium with 1.5% (w/v) agarose] with 50 µg/mL kanamycin to select the entry clone and ampicillin to select the expression clone, respectively. After incubation for 12 h at 37°C, single clones were isolated and the success of cloning was controlled by RT-PCR. Isolated clones were replicated in 7 mL LB medium for 12 h at 37°C.

As a control for the successful cloning of the hMATE1 vector constructs, RT-PCR was performed. dNTPs (10 nm), 5 µL 10x buffer, 20 pmol hMATE1 forward primer (5′ tcatgctgtgcatggagtggtg 3′ (chosen here to generate a product of approx. 500 bp), 20 pmol reverse primer (5′ agatgatgatccctgaccacaga 3′ (9), and 2 µL of Taq polymerase were filled up with nuclease-free water to a volume of 50 µL. The reaction was carried out in a peltier thermal cycler (PTC-200, MJ Research Inc., St. Bruno, QC, Canada) starting with a denaturation step at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30 s, primer annealing at 58.6°C for 30 s, and elongation at 72°C for 1 min. The last step was an elongation for 5 min at 72°C. PCR products were separated by gel electrophoresis on a gel of 1.5% agarose in Tris borate EDTA (TBE) buffer (w/v) with 0.05% (w/v) ethidium bromide at 100 mV.

Stable transfection of hMATE1 into HEK293 cells

The expression vector pEF5/FRT/V5/hMATE1 was stably transfected into Flp-In-TREx-HEK293-cells following the protocol from Invitrogen. At first, host cells containing Flp Recombination Target (FRT) site in their genome were generated. hMATE1 expression cells were subsequently generated. To this end, the pEF5/FRT/V5/hMATE1 expression vector and the pOG44 plasmid were transfected into Flp-In-TREx-HEK293 host cells. Recombination then occurred between the FRT site on the expression vector and the genome of the Flp-In-TREx-HEK293 host cells. Thereby, an initiation codon and a promoter for a hygromycin resistance gene on the expression vector were inserted so that transfected cells could be selected by hygromycin.

To transfect the Flp-In-TREx-HEK293 host cells with the pEF/FRT/V5/hMATE1 expression vector, 6×10^5 cells were seeded in 35 mM plates and allowed to grow for 12 h 0.35 µg expression vector and 3 µg pOG44 plasmid were prepared in 1 mL DMEM-HG-medium without FCS. A second solution contained 10 µL lipofectamin and 990 µL DMEM-HG-medium without FCS. After a 5 min incubation, solutions were mixed, incubated for 25 min and added to the cells. Cells were incubated for 5 h at 37°C. Medium was changed to a medium with FCS and cells were incubated for 24 h at 37°C. Cells were transferred to 10 mM dishes and incubated for 5 h at 37°C. 175 µg/mL hygromycin was added and cells were allowed to grow at 37°C.

Uptake measurements

Transport experiments with radioactively labeled substrates were performed as previously described (10). For these experiments, 2×10^5 Flp-In-TREx-HEK293-hMATE1 cells and non-expressing control cells were seeded per well in polylysine-coated 24-well plates and allowed to grow for 3 days. Reference transport solutions were prepared of 1 µM [^{3}H]MPP in Mammalian Ringer solution at pH 7.4 or 8.5, respectively, containing 130 mM NaCl, 4 mM KCl, 1 mM $CaCl_2$, 1 m< $MgSO_4$, 20 m< HEPES, 1 mM NaH_2PO_3 and 18 mM glucose per L of double distilled water (e.g., Figure 1). Test solutions for analysis of the transport activity of hMATE1 additionally contained 500 µM non-labeled MPP, TEA and quinine (Figure 1). Solutions for investigating the interaction of hMATE1 with antineoplastic agents contained in addition to [^{3}H]MPP and Mammalian Ringer 100 µM of the selected drug. The uptake time was 5 min and, as the activity of hMATE1 depends on a proton gradient, measurements were performed at pH 7.4 (Figures 3–5A) and 8.5. (Figures 3–5B). For the IC_{50} determination, [^{3}H]MPP uptake was measured in the presence of 0 to 300 µM of each antineoplastic agent (Figures 6 and 7).

Statistics

Data are presented as means and 95% confidence intervals of three experiments with three replicates. Significant differences in uptake rates were calculated by analysis of variance (ANOVA) followed by Tukey's test. p-Values of <0.001 are indicated by *** for the MATE1-expressing cells (°°° for the control cells), p<0.01 by ** and p<0.05 by *. For IC_{50} determinations, non-linear regression was performed. Analyses were generated using SAS (SAS Institute GmbH, Cary, NC, USA), Excel (Microsoft, Redmond, WA, USA), and Sigma Plot (Systat Software GmbH, San José, CA, USA).

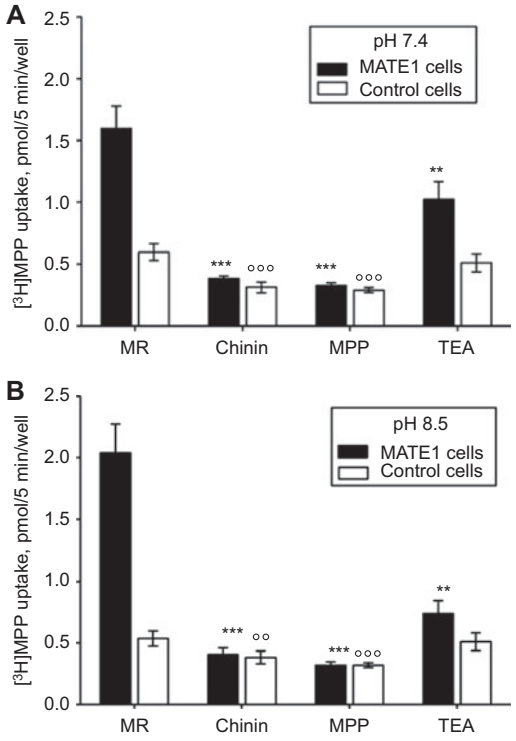

Figure 1 Functional expression of the hMATE1 protein in stably transfected HEK293 cells.
The uptake rate of 1 µM [^{3}H]MPP was measured in hMATE1 cells (black) and non-expressing control cells (white) at pH 7.4 (A) and 8.5 (B). Five min uptake rates are presented in pmol/5 min/well. Absolute uptake rates in Mammalian Ringer (MR) in hMATE1 stably transfected HEK293 cells were 1.60±0.68 pmol/5 min/well at pH 7.4 and 2.04±0.23 pmol/5 min/well at pH 8.5. Non-transfected control cells were 0.60±0.07 pmol/5 min/well at pH 7.4 and 0.54±0.06 pmol/5 min/well at pH 8.5. To inhibit the [^{3}H]MPP uptake by hMATE1 500 µM quinine, MPP or TEA were added, respectively. Data are means and 95% confidence intervals of three experiments with three repeats. * indicates the significant differences between stably transfected HEK293 cells and ° those between non-expressing control cells. ***p<0.001; °°°p<0.001 by Tukey's test.

Results

Transport activity of hMATE1 in stably transfected HEK293 cells

The hMATE1-mediated uptake of [^{3}H]MPP in stably transfected HEK293 cells was 1.60±0.68 pmol/5 min/well. Uptake in non-transfected control cells was 0.60±0.07 pmol/5 min/well as demonstrated in (Figure 1A). [^{3}H]MPP uptake into MATE1-expressing cells was significantly inhibited by 500 µM of unlabeled quinine, MPP and TEA by 77.2±3.0% (p<0.0001), 78.7±1.4% (p<0.0001) and 37.2±9.0% (p<0.001), respectively (Figure 1A). Because hMATE1 was described to be pH dependent, we examined MPP uptake and *cis*-inhibition studies at pH 8.5 (Figure 1B). The [^{3}H]MPP uptake at the experimentally generated outward proton gradient (pH 8.5 extracellular and approx. pH 7.4 intracellular) showed a 1.28-fold increase in [^{3}H]MPP uptake rate of 2.04±0.23 pmol/5 min/well as compared to pH 7.4. Non-expressing control cells exhibited an uptake rate of 0.54±0.06 pmol/5 min/well at pH 8.5, which was not different form the uptake at pH 7.4. The *cis*-inhibition experiments at pH 8.5 with 500 µM unlabeled quinine, MPP and TEA showed a significant (p<0.0001) inhibition of MPP uptake of 80.3±2.7%, 85.5±0.9% and 64.2±5.1%, respectively (Figure 1B).

Interaction of hMATE1 stably transfected in HEK293 cells with antineoplastic agents

Next, we evaluated the interaction of hMATE1 with several antineoplastic agents. We performed MPP uptake experiments into hMATE1-HEK293 cells and non-expressing control cells and measured the inhibition of hMATE1-mediated MPP uptake by antineoplastic agents at pH 7.4 as well as at pH 8.5. The interaction of hMATE1 with alkylating agents (Figure 2A, B), antibiotics and antimetabolites (Figure 3A, B), and topoisomerase inhibitors, mitosis inhibitors and a hormone receptor antagonist (Figure 4A, B) was determined. The results are presented as a percentage of MPP uptake by hMATE1-expressing cells treated with Mammalian Ringer without antineoplastic agents. From the alkylating agents, bendamustine was the only member that significantly inhibited hMATE1-facilitated MPP uptake. At pH 7.4, the uptake rate was inhibited by 26.6±7.7% with p<0.01 and at pH 8.5 by 51.0±5.2% with a significance value of p<0.001 (Figure 2A, B). Further alkylating agents, busulfan, chlorambucil, cyclophosphamide, ifosfamide, melphalan, thiotepa, treosulfan and trofosfamide did not show any significant inhibition of [^{3}H] MPP uptake.

We also investigated the interaction of hMATE1 with antibiotics and antimetabolites. The antibiotic mitoxantrone strongly inhibited the MPP uptake into hMATE1-HEK293 cells by 73.2±2.9% at pH 7.4 and by 84.1±1.8% at pH 8.5, respectively, with a high significance level of p<0.001 (Figure 3A, B). In addition, the antibiotic doxorubicin inhibited [^{3}H]MPP uptake into hMATE1-HEK293 cells at pH 7.4 by 37.4±6.9% and at pH 8.5 by 78.1±2.4% (both at p<0.001). There was also a significant inhibition by these antineoplastic agents in control cells but this effect was smaller than that in hMATE1 cells. The antibiotic mitomycin C did not exhibit an inhibition of MPP uptake into hMATE1-expressing cells.

Of the class of antimetabolites, only fluoradenine significantly inhibited MPP uptake into MATE1-expressing cells by 22.9±11.1% at pH 7.4 and by 23.3±8.5% at pH 8.5, respectively (p<0.05, Figure 3A, B). 5-Fluorouracil, cladribine, cytarabine, gemcitabine, methotrexate and mitomycin C did not show any specific interaction with hMATE1.

The topoisomerase inhibitor irinotecan significantly inhibited MPP uptake into hMATE1-HEK293 cells by 72.6±3.3% at pH 7.4 and by 78.1±2.6% at pH 8.5, respectively, with p<0.001 (Figure 4A, B). A second topoisomerase inhibitor, etoposide, showed a hMATE1 specific and significant inhibition of MPP uptake by 34.1±10.5% at pH 7.4 (p<0.01) and of 59.3±6.5% at pH 8.5 (p<0.001) (Figure 4A, B). The interaction of hMATE1 with inhibitors

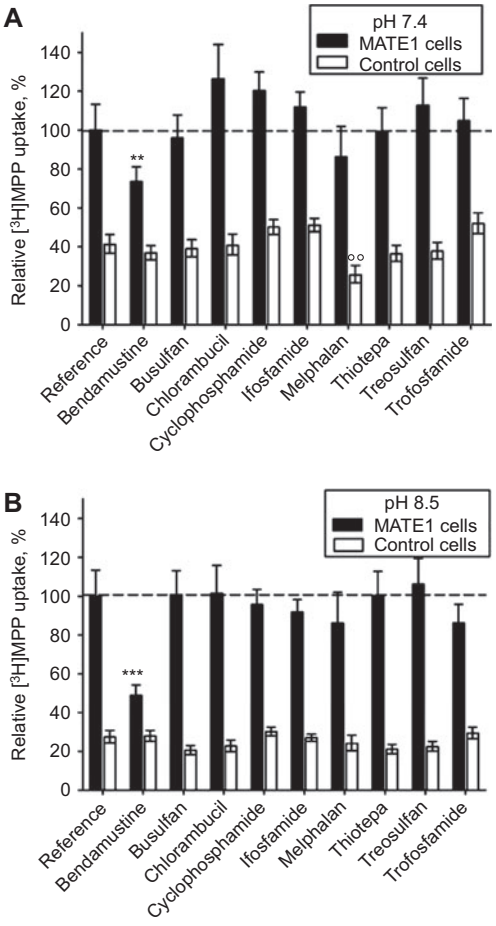

Figure 2 Interaction of hMATE1 with alkylating agents in stably transfected HEK293 cells.
The 5 min uptake rate of 1 µM [^{3}H]MPP was measured in hMATE1 cells (black) and non-expressing control cells (white) at pH 7.4 (A) and 8.5 (B). Uptakes are given in percent of the rate in Mammalian Ringer in hMATE1 cells (reference) and 100% uptake is labeled (dashed line). Absolute uptake rates in MR were 1.49±0.19 pmol/5 min/well at pH 7.4 and 1.94±0.26 pmol/5 min/well at pH 8.5, respectively. 100 µM cytostatic were added. Data are means and 95% confidence intervals of three experiments with three repeats. * indicates significant differences between stably transfected HEK293 cells and ° indicates those between non-expressing control cells. ***p<0.001; **p<0.01; °°p<0.01 by Tukey's test.

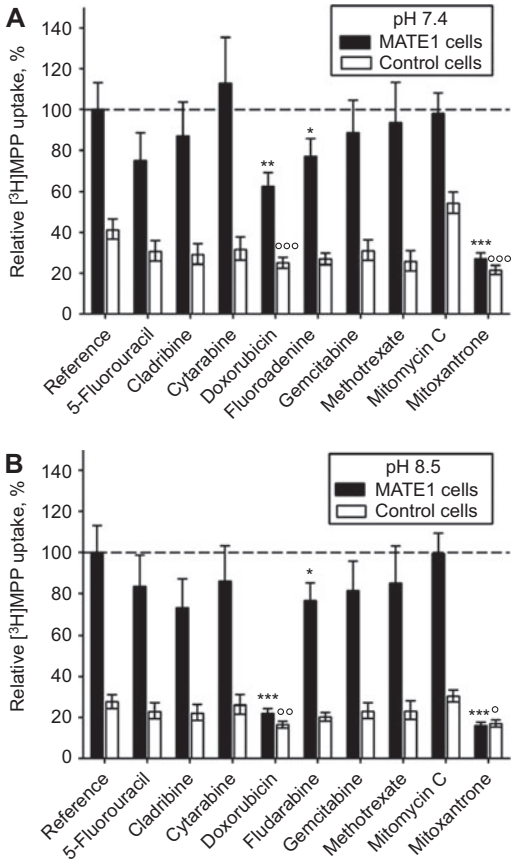

Figure 3 Interaction of hMATE1 with antibiotics and antimetabolites in stably transfected HEK293 cells.
The uptake of 1 µM [^{3}H]MPP was measured in hMATE1 cells (black) and non-expressing control cells (white) at pH 7.4 (A) and 8.5 (B). Uptake rates are given in percent of the rate in Mammalian Ringer in hMATE1 cells (reference) and 100% uptake is labeled (dashed line). Absolute uptake rates in MR were 1.49±0.19 pmol/5 min/well at pH 7.4 and 1.94±0.26 pmol/5 min/well at pH 8.5, respectively. 100 µM cytostatic were added. Data are means and 95% confidence intervals of three experiments with three repeats. * indicates significant differences between stably transfected HEK293 cells and ° indicates those between non-expressing control cells. ***p<0.001; **p<0.01; *p<0.05; °°°p<0.001; °°p<0.01; °p<0.05 by Tukey's test.

of mitotic spindle formation was also examined. All antineoplastic agents out of this group inhibited MPP uptake significantly only at pH 8.5 by 29.7±8.1% (vinblastine), 30.1±8.3% (vincristine) and 29.6±6.3% (vindesine) with a significance level of p<0.001. Vinblastine and vindesine inhibited MPP uptake only into MATE1-expressing cells, vincristine inhibited uptake also into control cells. For paclitaxel there was no significant inhibition of MPP transport. The hormone receptor antagonist tamoxifen showed a significant inhibition by 39.9±10.4% at pH 7.4 and a slight inhibition by 15.5±10.7% at pH 8.5 with p<0.001 and p<0.01, respectively.

Concentration dependent inhibition of hMATE1-mediated MPP uptake by mitoxantrone and irinotecan

Two antineoplastic agents, mitoxantrone and irinotecan, inhibited at a concentration of 100 µM hMATE1-mediated [^{3}H]MPP uptake by more than 50% at pH 7.4 and pH 8.5 (Figures 3 and 4). To elucidate the affinity of hMATE1 for these antineoplastic agents we measured the concentration dependent inhibition of hMATE1-mediated MPP uptake for mitoxantrone and irinotecan. The calculated IC_{50} values for the inhibition of [^{3}H]MPP uptake by mitoxantrone were 7.8±2.9 µM at pH 7.4 and 0.6±0.3 µM at pH 8.5, respectively (Figure 5A, B). For irinotecan, we calculated IC_{50} values of 4.4±2.3 µM at pH 7.4 and 1.1±0.7 µM at pH 8.5, respectively (Figure 6A, B).

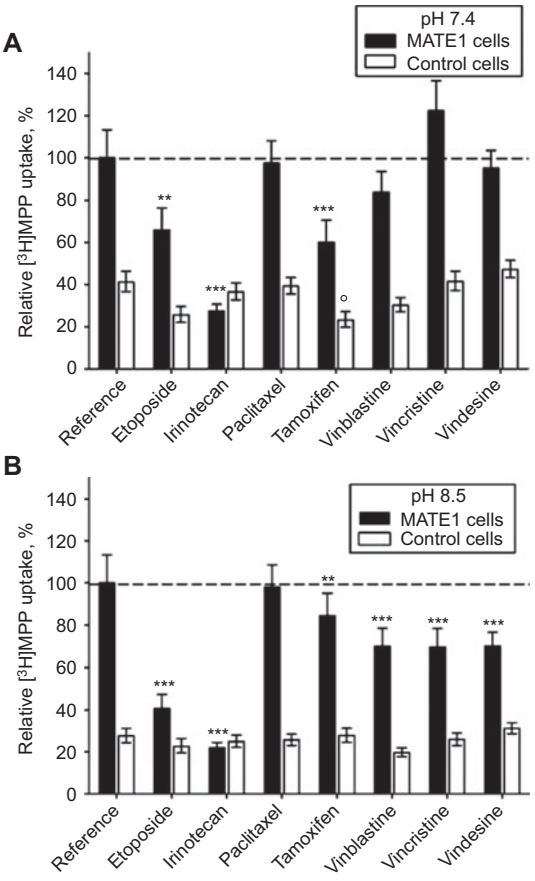

Figure 4 Interaction of hMATE1 with topoisomerase inhibitors, mitose inhibitors and hormones in stably transfected HEK293 cells. The 5 min uptake of 1 µM [^{3}H]MPP was measured in hMATE1 cells (black) and non-expressing control cells (white) at pH 7.4 (A) and 8.5 (B). Uptake rates are given in percent of the rate in Mammalian Ringer in hMATE1 cells (reference) and 100% uptake is labeled (dashed line). Absolute uptake rates in MR were 1.49±0.19 pmol/5 min/well at pH 7.4 and 1.94±0.26 pmol/5 min/well at pH 8.5, respectively. 100 µM cytostatic were added. Data are means and 95% confidence intervals of three experiments with three repeats. * indicates significant differences between stably transfected HEK293 cells and ° indicates those between non-expressing control cells. ***$p<0.001$; °$p<0.05$ by Tukey's test.

Discussion

The resistance of tumor cells and drug-drug interactions are two main problems of pharmacological therapy. The aim of this study was to investigate whether the SLC protein, hMATE1, could play a role in the interaction with antineoplastic agents and thereby in drug-drug interactions. hMATE1 is highly expressed in the brush-border membrane of proximal tubular cells and the bile canalicular membrane of hepatocytes. In the kidney, MATE1 is responsible for the exit of organic cations across the apical membrane (2, 3). hMATE1 was described to be an organic cation-proton exchanger. Thus, the driving force for hMATE1-mediated transport of organic cations or zwitterions is a proton gradient. Because the primary urine is acidic, hMATE1 functions in proximal tubule cells rather as an efflux than as an uptake transporter. Nevertheless, the bidirectional transport mode of hMATE1 for organic cations across the plasma membrane, depending on a proton gradient, is well established (1, 2). A few studies demonstrated the interaction of hMATE1 in the uptake mode with several drugs (2, 11–13).

Up to now, very little was known about the interaction of hMATE1 with antineoplastic agents. Meyer zu Schwabedissen et al. described an interaction of mitoxantrone with hMATE1 (8). A hMATE1-mediated transport of topotecan was described by Tanihara et al. 2007 (2). Furthermore, it has been found that hMATE1 transports the platin analogues cisplatin (weakly) and oxaliplatin, in vitro (7). Recently, Nakamura et al. have shown that cisplatin-induced nephrotoxicity was increased in MATE1-knockout mice (14). These findings indicate that hMATE1 plays an important role in the efflux of antineoplastic agents and that it is necessary to evaluate the interaction of hMATE1 with further antineoplastic agents.

Yonezawa et al. showed an inhibition of OCT1, OCT2, OCT3 and hMATE1-mediated TEA uptake by cisplatin and oxaliplatin (7). Furthermore, they demonstrated cisplatin and oxaloplatin induced cytotoxicity in cells expressing OCT1, OCT2, OCT3 and MATE1 (7). A comparable cisplatin and oxaloplatin induced cytotoxicity in cells expressing OCTs was reported by Zhang et al. (15). These studies document indirectly OCTs and MATE1 mediated transport of these antineoplastic agents (7, 15).

In this study, we examined the interaction of hMATE1 with 25 antineoplastic agents belonging to different classes of agents. For this purpose, we generated HEK293 cells stably transfected with hMATE1. Uptake experiments were performed at the physiological pH 7.4 and additionally at pH 8.5 to elevate the uptake rate by a higher outward-directed proton gradient. Compared to non-transfected control cells, we found a 2-fold and 4-fold MPP (1 µM) uptake rate into hMATE1-transfected cells after 5 min at pH 7.4 and pH 8.5, respectively. Tanihara et al. found an almost 9-fold uptake rate with 4 nm MPP after 15 min at pH 8.4. These data cannot be directly compared because of the difference in the concentration of MPP and the 3-fold longer uptake time (2).

It is known that tetraethylammonium (TEA) is a substrate of hMATE1 (2, 16). Tanihara et al. found a 2-fold higher uptake of MPP in comparison to TEA and K_m values of 0.1 mM for MPP and 0.38 mM for TEA but no interaction of hMATE1 with quinine (2). To further characterize our hMATE1-transfected cells we performed inhibition studies. The hMATE1 facilitated [^{3}H]MPP uptake was inhibited up to 86% by 500 µM unlabeled MPP and 61% by TEA at pH 8.5. In contrast to the results reported by Tanihara et al. (2), we observed an almost 80% inhibition of hMATE1-mediated MPP uptake by quinine.

To elucidate the interaction of hMATE1 with antineoplastic agents we performed pH dependent *cis*-inhibition of [^{3}H]MPP uptake by several antineoplastic agents. The alkylating agent bendamustine, the antibiotics doxorubicin and mitoxantrone, the topoisomerase inhibitors etoposide and irinotecan, and the hormone receptor antagonist tamoxifen showed a significant

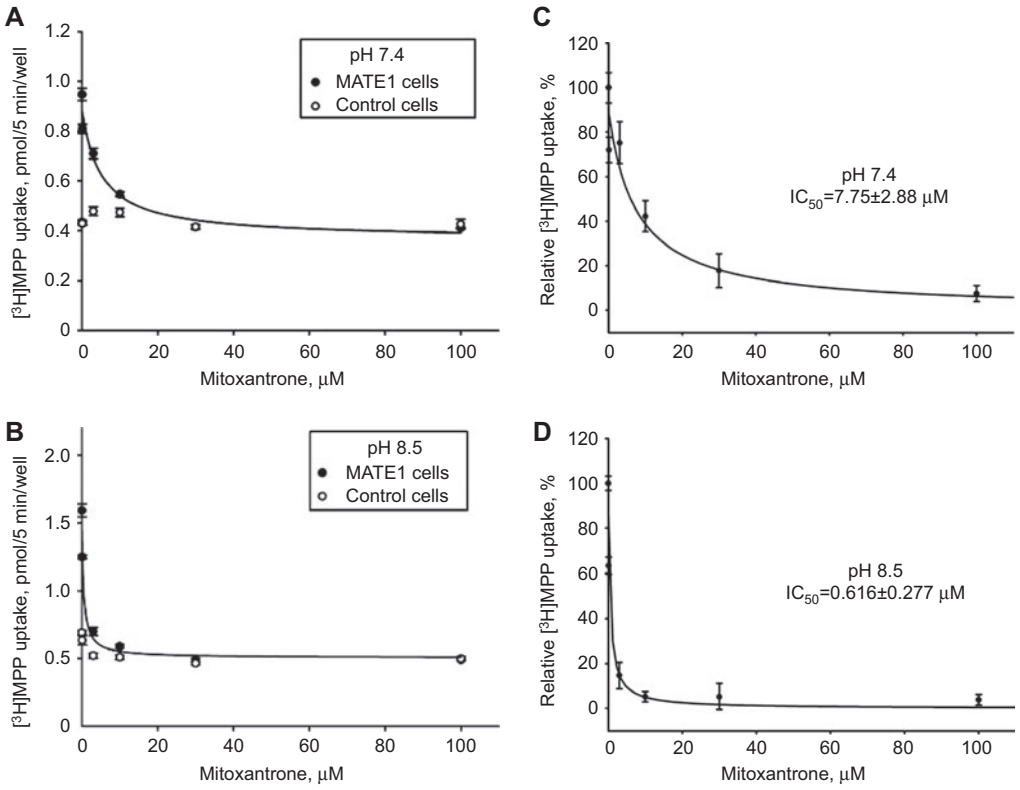

Figure 5 IC_{50} determination for the interaction of hMATE1 with mitoxantrone.
The 5 min uptake of 1 µM [^{3}H]MPP was measured in hMATE1 cells (black) and non-expressing control cells (white) at pH 7.4 (A) and 8.5 (B) with a mitoxantrone concentration of 0 to 100 µM. Absolute uptake rates in MR were 0.95±0.04 pmol/5 min/well at pH 7.4 and 1.59±0.09 pmol/5 min/well at pH 8.5, respectively. (C, D) The hMATE1 dependent uptake rate adjusted for the uptake rate in control cells. Uptake rate in Mammalian Ringer was set at 100%. IC_{50} values were determined by non-linear regression. Data are one of three experiments with three repeats.

inhibition of [^{3}H]MPP uptake into hMATE1-HEK293 cells at pH 7.4. An interaction of molecules with such different chemical structures and charge matches to the polyspecific substrate specificity of hMATE1 (2, 6).

A change in the pH of the transport solution from 7.4 to 8.5 led to an increased inhibition by the antineoplastic agents mentioned above and additional inhibition by antimetabolites and mitose inhibitors. This could be due to a change of the molecules' charge or structures. Irinotecan molecules, for example, change from the lactone form nearly completely into the carboxylate form when the pH is increased from 7.4 to 8.5 (17). In general, if there is a change of charge, molecules would turn more negative or less positive in their net charge. hMATE1 was described not only to interact with and transport organic cations but also organic anions. Tanihara et al. found, for example, a hMATE1-mediated transport of estrone-3-sulfate with a K_m of 0.47 mM compared to 0.38 mM for the organic cation metformin (2). Consequently, this could explain the higher inhibition by antineoplastic agents at pH 8.5.

As all inhibitions by unlabeled substrates and antineoplastic agents were stronger at pH 8.5 than at pH 7.4, it is more probable that the pH directly has an influence on the characteristics of hMATE1. The only exception was tamoxifen, which at pH 8.5 showed less inhibition than at pH 7.4. Tamoxifen has a pK_a value of 8.5 as determined by the SPARC online calculator. At pH 8.5 or above, most of the tamoxifen molecules are uncharged and therefore tamoxifen could have a lower inhibitory potency for hMATE1 than at pH 7.4.

Irinotecan and mitoxantrone exhibited the strongest inhibition of [^{3}H]MPP uptake into hMATE1-HEK293 cells. Interaction of hMATE1 with mitoxantrone was already described for the uptake of radioactively labeled metformin and TEA with an IC_{50} value of 4.4 µM for metformin and 5.2 µM for TEA at pH 7.4 (8). We observed a comparable IC_{50} value of 7.8 µM for the uptake of [^{3}H]MPP at pH 7.4. The interaction of hMATE1 with irinotecan has to our knowledge not been demonstrated up to now. Our results show an inhibition of hMATE1-mediated MPP uptake by irinotecan and the calculated IC_{50} value is approximately 4.4 µM.

At pH 8.5 we saw a stronger inhibition and decreased IC_{50} values of 1.1 µM for irinotecan and 0.6 µM for mitoxantrone. For irinotecan, this could be due to a structure change from the lactone form into its carboxylate form at pH 8.5 as mentioned above (17).

Although inhibition of MATE1 does not prove transport, our data definitively verify the inhibition of the activity of hMATE1 by antineoplastic agents. As a consequence, these

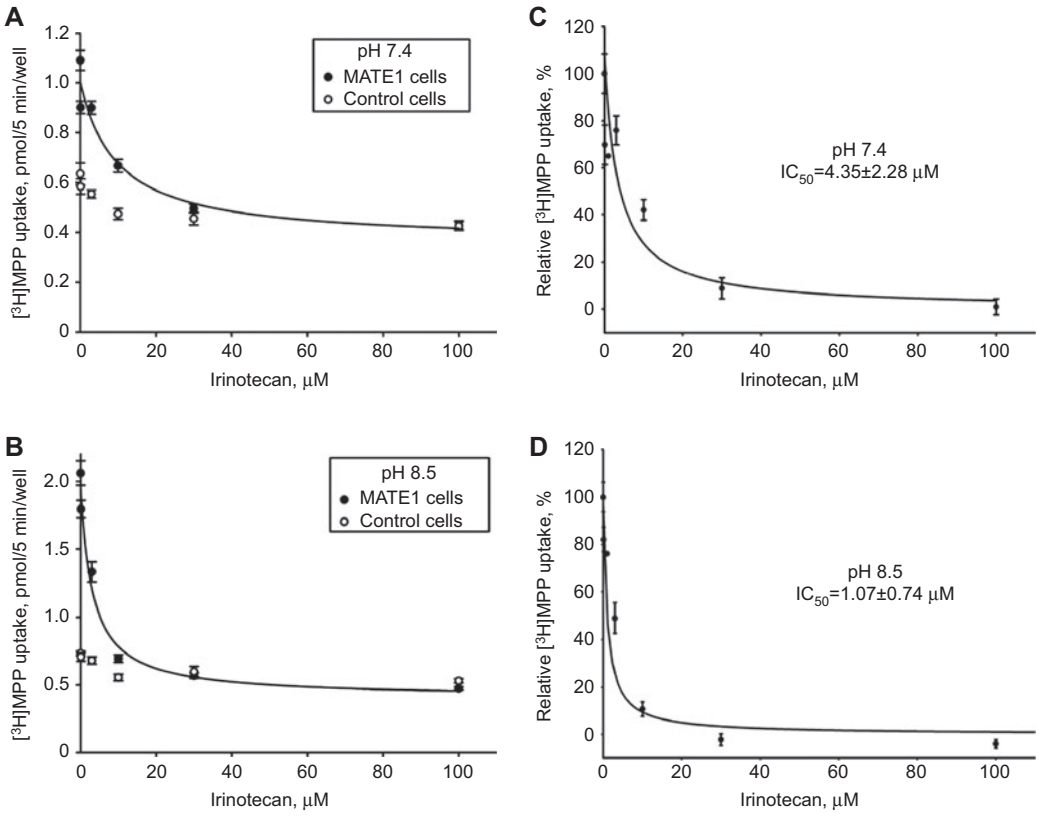

Figure 6 IC_{50} determination for the interaction of hMATE1 with irinotecan.
The 5 min uptake of 1 μM [^{3}H]MPP was measured in hMATE1 cells (black) and non-expressing control cells (white) at pH 7.4 (A) and 8.5 (B) with an irinotecan concentration of 0 to 100 μM. Absolute uptake rates in MR were 1.09±0.12 pmol/5 min/well at pH 7.4 and 2.06±0.08 pmol/5 min/well at pH 8.5, respectively. (C, D) The hMATE1 dependent uptake rate adjusted for the uptake rate in control cells. Uptake rate in pure Mammalian Ringer was set at 100%. IC_{50} values were determined by non-linear regression. Data are one of three experiments with three repeats.

antineoplastic agents might be responsible for pharmacologically relevant drug-drug interactions (DDI) in kidneys, liver and skeletal muscle cells where hMATE1 is expressed. We speculate that an inhibition of hMATE1-facilitated excretion of endogenous compounds, toxins and drugs from the body via kidneys and liver might cause general side effects. Along the same lines, the inhibition of MATE1-dependent creatinine release from skeletal muscle cells might cause cell toxicity. The influence of DDI on hMATE1 was functionally demonstrated by the inhibition of hMATE1-mediated metformin transport by cimetidine in renal epithelial cells (18). Furthermore, Kusuhara et al. described the inhibition of hMATE1 by pyrimethamine and a subsequent decrease of the renal clearance of metformin and creatinine as well as a reduction of metformin uptake into canalicular membrane vesicles mediated by hMATE1 (19).

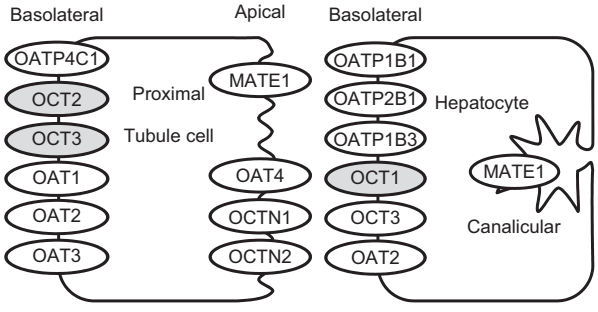

Figure 7 Localization of proteins in proximal tubular cells and hepatocytes.
Members of the families SLC21, SLC22 and hMATE1 are shown. SLCs known to share antineoplastic agents with hMATE1 are shown in grey.

It has to be evaluated to which degree the antineoplastic agents interacting with hMATE1 can lead to DDI and influence the renal or hepatic clearance of other endogenous metabolites and exogenous drugs, toxins and furthermore the secretion of creatinine from skeletal muscle cells.

Efflux of antineoplastic agents from the cells mediated by hMATE1 could in theory, cause resistance of tumor cells. Due to an acidic interstitial microenvironment in solid tumors as described in several studies (20–22), an inwardly directed proton gradient exists driving antineoplastic agents out of the cells if hMATE1 is expressed and if it really translocates these drugs.

Table 1 Antineoplastic agent interacting with hMATE1 as well as with SLC22 transporter family.

Antineoplastic agent interacting with hMATE1	OCT1	OCT2	OCT3	OCT6	OAT1	OAT3	OCTN1
Mitoxantrone	+	+	+	nd	no	+	+
Irinotecan	+	+	+	nd	nd	nd	nd
Doxorubicine	nd	nd	no	+	nd	nd	+
Etoposide	+	+	+	nd	nd	nd	nd
Bendamustine	no	no	no	nd	no	+	nd
Tamoxifen	nd	nd	+	nd	+	no	nd

SLC22 family interacting with antineoplastic agents (10, 30–32) that have also been shown to interact with hMATE1 in this study. +, significant interaction; no, no significant interaction; nd, not determined.

The excretion of various substrates by the kidney and liver is known to be a two-step process, with an uptake and an efflux step. Several studies documented that hMATE1 collaborates as an efflux transporter with several uptake transporters of the SLC transporter family as schematically shown in Figure 7 [modified from (23–26)]. The renal clearance of metformin is facilitated by an interplay between OCT2 and hMATE1 (27). Recently, in vivo studies in rat and monkey showed a collaborative trans-cellular transport of the antibiotic PN-288034 by OAT3 and hMATE1 (28).

Nakamura et al. have shown a potentiation of cisplatin-induced nephrotoxicity in MATE1-knockout mice (14). In contrast, it was shown by Filipski et al. that OCT2 knockout mice exhibit a decreased cisplatin-induced nephrotoxicity (29). These results indicate a collaborative transport of cisplatin via OCT2 and MATE1 in proximal tubule cells and elucidate the importance of MATE1 interplay with further SLC proteins.

The interactions of antineoplastic agents with the members of the SLC22 uptake transporters were reported recently by Gupta et al. (30) and Table 1 shows antineoplastic agents that inhibit hMATE1 as well as some members of the SLC22 family. OCT1, OCT2 and OCT3 were reported to interact with irinotecan as well as with mitoxantrone (30) as does hMATE1 (this study). Thereby, OCT1 could take up irinotecan and mitoxantrone into hepatocytes, and hMATE1 could eliminate these antineoplastic agents into bile (Figure 7). In the kidney there could be a collaboration of OCT2 and OCT3 at the basolateral side of the proximal tubule cells and hMATE1 at the luminal side as shown in Figure 7. The members OATP1B1 and OATP1B3 of SLCO21 family are highly expressed in the liver (Figure 7) but do not share an interaction with the antineoplastic agents that we tested for hMATE1. However, they are potential collaborators with hMATE1 in the liver and kidney.

In summary, we examined the interaction of hMATE1 with 25 well-known antineoplastic agents that are used in the chemotherapy of tumours. We observed a significant interaction with the antineoplastic agents: irinotecan, mitoxantrone, etoposide, doxorubicin, tamoxifen, bendamustine, fludarabine, vinblastine and vindesine with hMATE1. Furthermore, we determined the pH dependent IC_{50} values of hMATE1 for mitoxantrone and irinotecan. The observed interactions could give rise to drug-drug interactions and if hMATE1 transports the antineoplastic agents, to a decrease in the chemosensitivity of tumor cells expressing MATE1. Further studies are needed to test whether antineoplastic drugs are indeed translocated or are only acting as inhibitors.

Conflict of interest statement

Authors' conflict of interest disclosure: The authors stated that there are no conflicts of interest regarding the publication of this article.
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