

An *in vitro* study on interaction of anisodine and monocrotaline with organic cation transporters of the SLC22 and SLC47 families

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[ABSTRACT] Current study systematically investigated the interaction of two alkaloids, anisodine and monocrotaline, with organic cation transporter OCT1, 2, 3, MATE1 and MATE2-K by using *in vitro* stably transfected HEK293 cells. Both anisodine and monocrotaline inhibited the OCTs and MATE transporters. The lowest IC₅₀ was 12.9 μmol·L⁻¹ of anisodine on OCT1 and the highest was 1.8 mmol·L⁻¹ of monocrotaline on OCT2. Anisodine was a substrate of OCT2 ($K_m = 13.3 \pm 2.6 \mu\text{mol}\cdot\text{L}^{-1}$ and $V_{\text{max}} = 286.8 \pm 53.6 \text{ pmol}/\text{mg protein}/\text{min}$). Monocrotaline was determined to be a substrate of both OCT1 ($K_m = 109.1 \pm 17.8 \mu\text{mol}\cdot\text{L}^{-1}$, $V_{\text{max}} = 576.5 \pm 87.5 \text{ pmol}/\text{mg protein}/\text{min}$) and OCT2 ($K_m = 64.7 \pm 14.8 \mu\text{mol}\cdot\text{L}^{-1}$, $V_{\text{max}} = 180.7 \pm 22.0 \text{ pmol}/\text{mg protein}/\text{min}$), other than OCT3 and MATE transporters. The results indicated that OCT2 may be important for renal elimination of anisodine and OCT1 was responsible for monocrotaline uptake into liver. However neither MATE1 nor MATE2-K could facilitate transcellular transport of anisodine and monocrotaline. Accumulation of these drugs in the organs with high OCT1 expression (liver) and OCT2 expression (kidney) may be expected.

[KEY WORDS] Anisodine; Monocrotaline; Organic cation transporter; OCT; MATE

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Introduction

Anisodine, a natural tropane alkaloid, was isolated from native Chinese plant *Anisodus tanguticus* (Maxim.) Pascher. As a muscarinic receptor antagonist, anisodine has various pharmacological activities such as anti-spasm, anti-tremor and so on. In clinic, anisodine has been used for cerebral vascular occlusive disease and rescuing patients from cerebral hypoxia due to its beneficial effects on central nerve system [1]. Besides that, it was developed as a traditional Chinese medicine for ophthalmology treatment by improving intraocular micro-circulation [2]. Several studies have revealed the metabolic

pathway of anisodine *in vivo* and *in vitro* [3-4], but the knowledge of its transcellular transport is still missing.

Toxic pyrrolizidine alkaloid monocrotaline was extracted from plant *Crotalaria sessiliflora*. Monocrotaline could lead to hepatic sinusoidal obstruction syndrome (HSOS). Indeed ingested monocrotaline undergoes bioactivation mediated by CYP3A4 and its active metabolite dehydromonocrotaline is the main reason for toxicity [5-6]. Dehydromonocrotaline could alkylate DNA and cytoskeletal component actin [7-8], and it has been recently reported to cause significant glutathione depletion and form pyrrole-protein complex in hepatic sinusoidal endothelial cells [9]. Health problems may occur when people are exposed to foods contaminated with toxic herbal elements such as monocrotaline, the knowledge of its transcellular mechanism would provide us with a prospective way to avoid toxicity more efficiently.

Both anisodine and monocrotaline are weak bases (Fig. 1). This naturally leads them to be potential inhibitors and substrates of the organic cation transporters (OCT). The member OCT1, OCT2, OCT3 from solute carrier (SLC) family 22 and the member MATE1, MATE2-K from family SLC47 are membrane proteins which could facilitate the absorption, distribution and elimination of endogenous or exogenous

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cationic compounds. Substrates of organic cation transporters are often overlapped [10–11]. In fact, such proteins are bi-directional transporters, but their different expression location and membrane potential result in the functional diversity: OCT1, 2 and 3 usually act as uptake transporters while MATE1 and MATE2-K act as efflux transporters in liver or kidney [10, 12]. OCT1 and MATE1 are mainly expressed at basolateral side and apical side of hepatocytes, respectively. OCT2, MATE1, and MATE2-K are highly expressed in proximal tubule epithelial cells: OCT2 at the basolateral side, MATE1 and MATE2-K at brush-border side. OCT3 has a broad distribution in different organs in the human body.

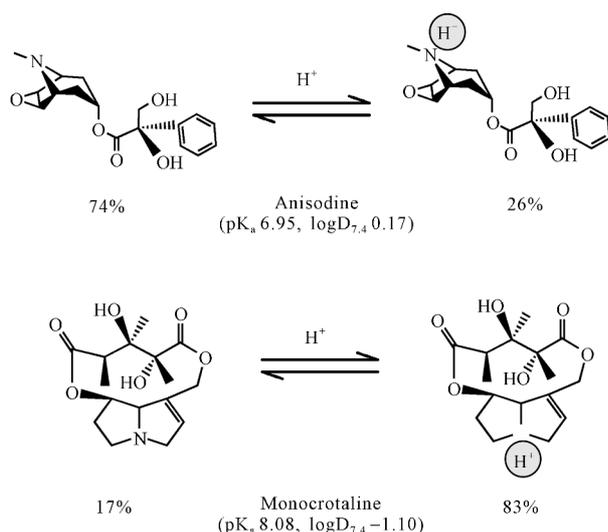


Fig. 1 Chemical structures of anisidine and monocrotaline and the equilibrium between the protonated and non-protonated form at pH 7.4. Predicted pK_a and logD_{7.4} values were showed in brackets. The prediction data was determined by MarvinView (version 6.1.0)

A previous report demonstrated that OCT1 was a key transporter for monocrotaline hepatic uptake with K_m of 25 $\mu\text{mol}\cdot\text{L}^{-1}$, monocrotaline markedly inhibited OCT1 with the IC_{50} of 5.52 $\mu\text{mol}\cdot\text{L}^{-1}$ [13]. Furthermore, inhibition of OCT1 decreased the hepatic toxicity of monocrotaline *in vitro*. However the interaction of monocrotaline with other organic cation transporters are unknown.

This study was the first time to investigate the interaction of two alkaloids, anisidine and monocrotaline, with organic cation transporters OCT1, 2, 3, MATE1 and MATE2-K by using the artificial membrane and the stably transfected HEK293 cells. Results would reveal the role of organic cation transporters (OCTs and MATEs) on anisidine and monocrotaline's disposition. Parallel artificial membrane permeability assay (PAMPA) was conducted to evaluate compound's passive diffusion capacity. Subsequently inhibition and transport experiments on transporter-overexpressing HEK293 cells were performed to explore the affinity and transport capacity of organic cation transporters to the two alkaloid compounds *in vitro*.

Materials and Methods

Chemicals

Anisidine was purchased from the National Institutes for Food and Drug Control of China (Beijing, China). Monocrotaline as well as atropine, tyramine, cimetidine, carnitine were obtained from Sigma-Aldrich (Taufkirchen, Germany). Tritium labeled methyl-4-phenylpyridinium (³H-MPP⁺) was purchased from Hartmann Analytic (Braunschweig, Germany).

Parallel artificial membrane permeability assay

Anisidine and monocrotaline were prepared at 20, 100 and 500 $\mu\text{mol}\cdot\text{L}^{-1}$ in phosphate buffer saline (PBS) solution. Three-hundred microliter of compound solution was added into donor wells of pre-coated 96-well PAMPA plate, and 200 μL of PBS only was added into acceptor wells. Five hours later, incubation was stopped by separating donor and acceptor wells and samples from acceptor wells were pipetted out. Concentrations of anisidine and monocrotaline in acceptor wells were determined by API 4000 LC-MS/MS system. Membrane permeability (P_e) was calculated according to equations (1) and (2):

$$C_{\text{equilibrium}} = [C_D(t) \times V_D + C_A(t) \times V_A] / (V_D + V_A) \quad (1)$$

$$P_e = \frac{-\ln[1 - C_A(t)/C_{\text{equilibrium}}]}{A \times (1/V_D + 1/V_A) \times t} \quad (2)$$

where $C_D(t)$ was the final concentration in donor well, $C_A(t)$ was the final concentration in acceptor well. V_D , V_A , A and t were constants which present the volume of a donor well (0.3 mL), the volume of an acceptor well (0.2 mL), the area of each well (0.3 cm^2) and the incubation time (18 000 s), respectively.

Cells overexpressing organic cation transporters

OCT3-HEK293 was obtained from Prof. Koepsell and Dr. Gorboulev (University of Würzburg, Germany). HEK293 cells overexpressing OCT1 or OCT2 were previously established in our lab [14–15]. MATE1-HEK293 and MATE2-K-HEK293 were constructed by following the previous methods [16]. Template plasmids pcDNA5::MATE1 and pcDNA5::MATE2-K were obtained from Dr. Hagos and Dr. Kühne (PortaCellTech, Göttingen, Germany). The forward and reverse PCR primers for MATE1 were 5'-GCAGCGCGTACCGACATGGAAGCTCCTGAGGAGCCCGCG-3' and 5'-ACTTTCTTTGATATCCACGTCCTGAATTCTGACATAGA-3', respectively; and for MATE2-K were 5'-GTGCCCCGGTACCAGGAATGGACAGCCTCCAGGACACA-3' and 5'-TATTTCCAAGATATCTTTGCTAGTGCTGGTGGCTAGGA-3', respectively. Primers were obtained from Eurofins Genomics (Munich, Germany). The PCR products were constructed with a clone vector pCR-XL-TOPO, subsequently target gene of transporter was re-sequenced and inserted into the expression vector pcDNA5.1. For the functional confirmation, 10 $\text{nmol}\cdot\text{L}^{-1}$ of ³H-MPP⁺ was applied as a model substrate. The uptake of ³H-MPP⁺ in transporter-overexpressing HEK293 cells and in mock cells was determined.

Cellular uptake assay

Six hundred thousand cells were seeded on a 12-well plate with pre-coated poly-D-lysine (1–4 kDa). Cells were confluent after 48 h under 37 °C with 5% CO₂. Assay buffer consisted of HBSS supplemented with 10 mmol·L⁻¹ HEPES. Initially cell culture medium was aspirated and cells were washed with 1 mL warm assay buffer. For the experiments of efflux transporters, MATE1-HEK293 and MATE2-K-HEK293 cells were pre-incubated with assay buffer supplemented with 30 mmol·L⁻¹ NH₄Cl for 30 min in order to reverse the direction of transport. Incubation buffer was replaced by 400 μL of assay buffer containing alkaloid or ³H-MPP⁺ with alkaloid to initialize the reaction. Reaction time was 2 min on OCT1-HEK293, OCT2-HEK293 and OCT3-HEK293 cells, and 1 min on MATE1-HEK293 and MATE2-K-HEK293 cells. Then cells were washed twice with 2 mL ice-cold assay buffer and lysed by 500 μL lysis buffer. Intracellular concentrations of anisodine and monocrotaline were determined by API 4000 LC-MS/MS system and intracellular ³H-MPP⁺ was determined by liquid scintillation counter (Zinsser Analytics, Germany). All experiments were conducted at least three times and quantitation results were normalized by total protein which obtained from BCA method.

HPLC-MS/MS quantitation

Intracellular accumulation of anisodine and monocrotaline were measured by HPLC-MS/MS system. Tyramine and atropine were used as internal standards for anisodine and monocrotaline, respectively. Two hundred microliter of cell lysate was evaporated under nitrogen flow and subsequently anisodine was condensed at the ratio of 1 : 3, monocrotaline at the ratio of 1 : 2 in 0.1% formic acid. A Brownlee SPP RP-Amide column (2.7 μm, 4.6 mm × 100 mm) with a C₁₈ ODS pre-column (4 mm × 2 mm, KJO-4282) were applied for chromatographic separation. Mobile phase consisted of organic phase (6 : 1, acetonitrile : methanol) and water phase, specifically 3% organic phase for anisodine quantitation and 15% organic phase for monocrotaline quantitation. The mass spectrometer was performed in positive MRM mode with *m/z* 320.1→156.0 for anisodine and *m/z* 326.2→120.2 for monocrotaline.

Data analysis

Predicted dissociation constant (pK_a) and distribution coefficient (logD_{7.4}) values were obtained from software MarvinView (version 6.1.0, ChemAxon Ltd., Budapest, Hungary); the half-maximal inhibitory concentrations (IC₅₀), the Michaelis-Menten constant (K_m) and the maximal transport rate (V_{max}) were determined by software SigmaPlot (version 12.5, Systat Software Inc., Erkrath, Germany), related formulas were given as below:

$$pK_a = pH + \log_{10} \frac{[\text{conjugated acid}]}{[\text{conjugated base}]} \quad (3)$$

$$\log D = \log \left(\frac{[\text{solute}]_{\text{octano}}^{\text{ionized}} + [\text{solute}]_{\text{octano}}^{\text{un-ionized}}}{[\text{solute}]_{\text{water}}^{\text{ionized}} + [\text{solute}]_{\text{water}}^{\text{un-ionized}}} \right) \quad (4)$$

$$y = \min + \frac{(\max - \min)}{1 + (x / IC_{50}) - \text{Hillslope}} \quad (5)$$

$$V = \frac{V_{\max} [S]}{K_m + [S]} \quad (6)$$

Results

Membrane permeability of anisodine and monocrotaline

Anisodine and monocrotaline are partially protonated under certain pH (Fig. 1). Twenty-six percent of anisodine and 83% of monocrotaline are in cation forms at pH 7.4 with logD values of 0.17 and -1.1, respectively. These *in silico* results indicated that monocrotaline has a lower permeability than anisodine and were confirmed in the parallel artificial membrane permeability assay (Fig. 2). Monocrotaline obtained a similar P_e value of (1.5 ± 0.1) × 10⁻⁶ cm·s⁻¹ with this of a known OCT1 and OCT2 substrate sulpride [P_e = (1.2 ± 0.3) × 10⁻⁶ cm·s⁻¹] [14]. The permeability of anisodine [P_e = (2.9 ± 0.3) × 10⁻⁶ cm·s⁻¹] was similar with which of reference compound 2-(2-methylquinolin-4-ylamino)-N-phenylacetamide [P_e = (3.0 ± 0.07) × 10⁻⁶ cm·s⁻¹] [17].

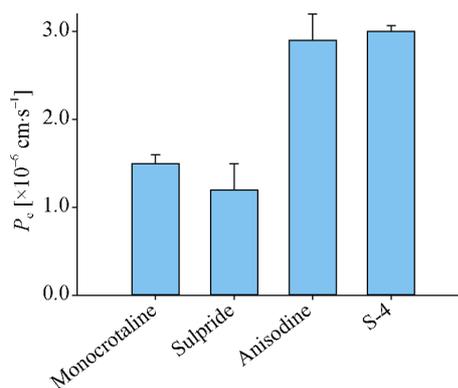


Fig. 2 Permeability of anisodine, monocrotaline, sulpride and S-4. The P_e values of anisodine and monocrotaline were determined by parallel artificial membrane permeability assay (PAMPA). All data are represented as means ± SEM (n = 3). Reference compounds sulpride and S-4 are compounds with medium permeability [14, 17]. S-4: 2-(2-methylquinolin-4-ylamino)-N-phenylacetamide

Inhibitory effects of anisodine and monocrotaline on OCTs and MATEs mediated uptake

The functions of these transporter-overexpressing cells were verified with the probe substrate MPP⁺. Comparing with mock cells, intracellular accumulation of 10 nmol·L⁻¹ ³H-MPP⁺ significantly increased in OCT1, OCT2, OCT3, MATE1 and MATE2-K overexpressing cells at 23-fold, 15-fold, 23-fold 36-fold and 22-fold, respectively (Fig. 3).

Inhibition assays were conducted by incubating the transporter-overexpressing cells with 5 nmol·L⁻¹ ³H-MPP⁺ in the presence of increasing concentrations of anisodine or monocrotaline. IC₅₀ values were calculated to compare inhibitory

potency of the two compounds to the different transporters. As shown in Fig. 4, both anisodine and monocrotaline caused a concentration dependent inhibition of MPP⁺ uptake. The lowest IC₅₀ value was obtained from anisodine on OCT1 (12.96 μmol·L⁻¹) which followed by OCT3, OCT2, MATE1 and MATE2-K (Table 1). Anisodine was 126 times more po-

tent at inhibiting OCT1 than MATE2-K. Weak affinity was not only observed on MATE2-K with anisodine, but also on OCT2 and OCT3 with monocrotaline (IC₅₀ > 1 mmol·L⁻¹). Interestingly, for monocrotaline the rank order of IC₅₀ values was much different from anisodine, as OCT1 < MATE2-K < MATE1 < OCT2 < OCT3.

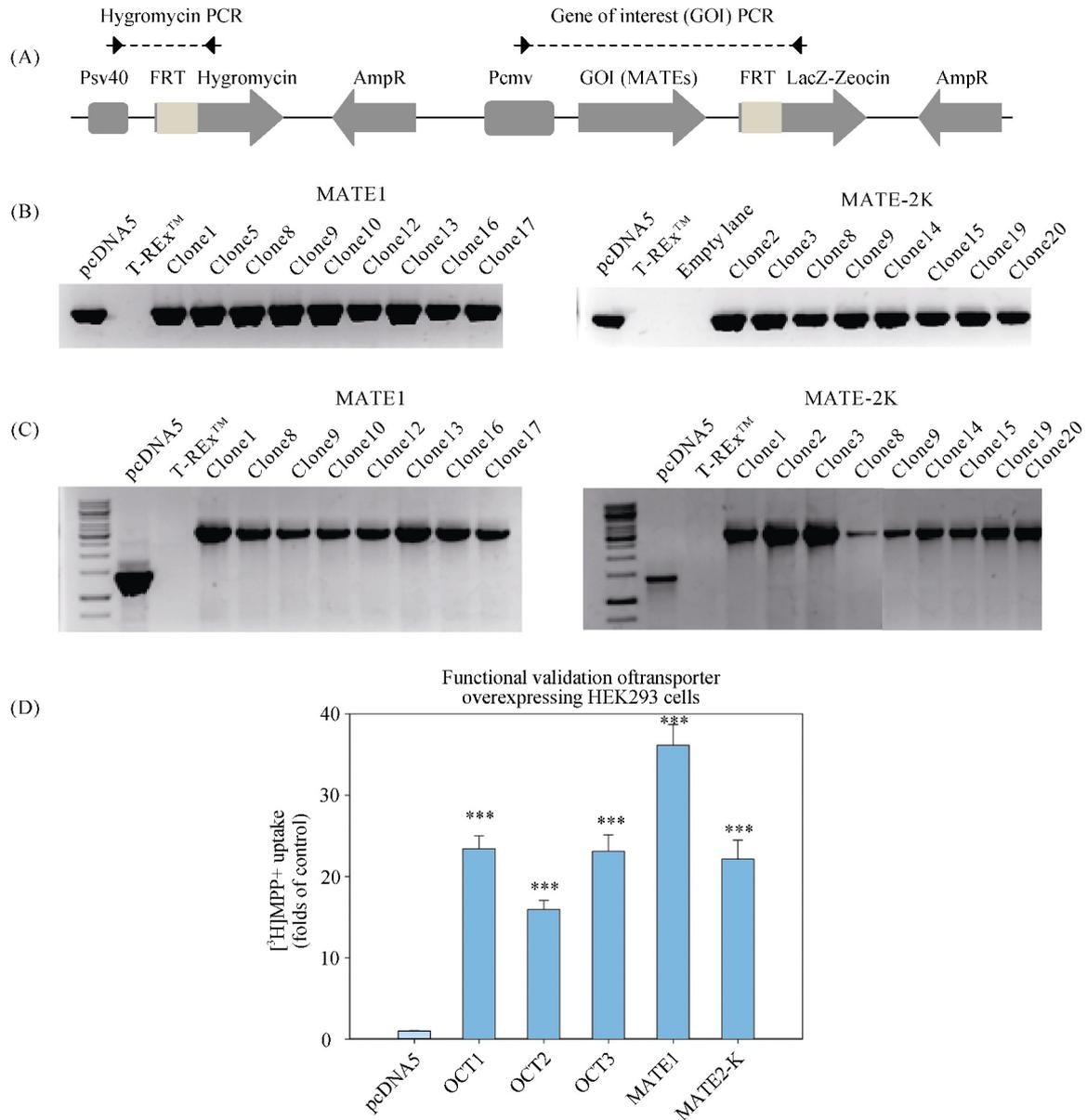


Fig. 3 Validation of transporter-overexpressing HEK293 cell models. Two regions of the genome of HEK293 cells by hygromycin PCR and gene of interest PCR (A). The expression plasmids were integrated at the genomic FRT site of T-REx™ cells, PCR detections of different colonies for amplification of a downstream of PSV40 promoter and upstream of the hygromycin resistance gene (B) and a downstream of PCMV promoter and upstream of the lacZ-Zeocin region (C) were performed. Clone 8 of MATE1 and clone 20 of MATE2-K were chosen for subsequent experiments. Functional validation of MATE1-overexpressing HEK293 cells and MATE2-K-overexpressing HEK293 cells (D). Uptake of 10 nmol·L⁻¹ ³H-MPP⁺ in mock cells (with empty vector transfected only) and in chosen transporter-overexpressing cells were determined. All data are represented as means ± SEM (n = 3). ***P < 0.001 vs control. ANOVA followed by post hoc analyses applying Tukey's Honestly Significant Different test

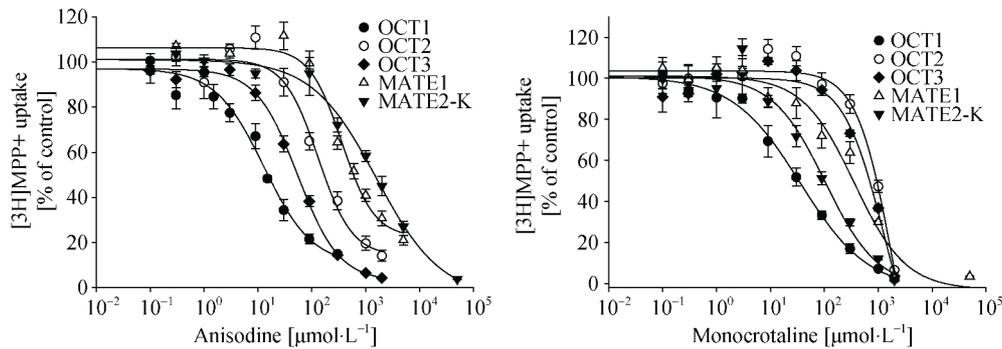


Fig. 4 Inhibition of MPP^+ uptake by anisidine (A) and monocrotaline (B). HEK293 cells stably overexpressing OCT1, OCT2, OCT3, MATE1, or MATE2-K were incubated for certain time (2 min for OCTs and 1 min for MATEs) with $5 \text{ nmol}\cdot\text{L}^{-1}$ $^3\text{H}\text{-MPP}^+$ in the presence of increasing concentration of the inhibitor. The uptake was presented as percentage of the uptake without inhibitor. The data are represented as means \pm SEM ($n = 3$)

Table 1 Half maximal inhibitory concentrations (IC_{50}) of anisidine and monocrotaline on organic cation transporters

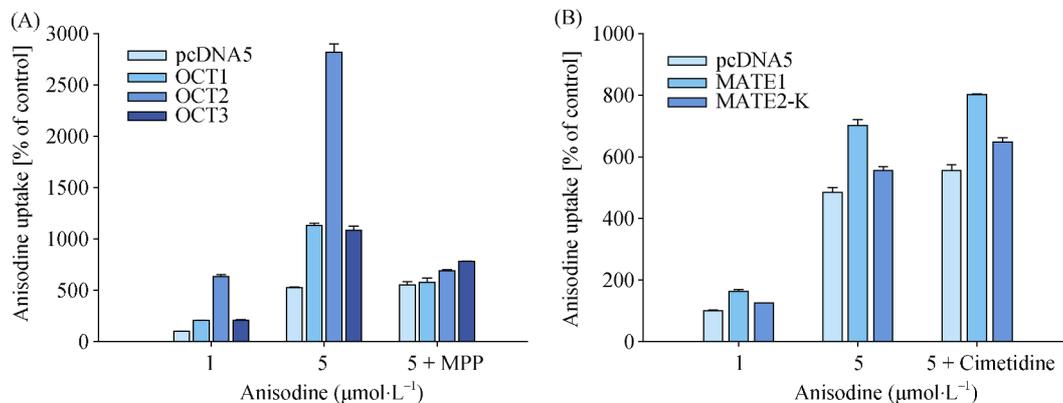
Compound	$\text{IC}_{50}/(\mu\text{mol}\cdot\text{L}^{-1})$				
	OCT1	OCT2	OCT3	MATE1	MATE2-K
Anisidine	12.9 ± 0.9	139.6 ± 15.2	54.7 ± 6.7	366.5 ± 46.2	1631.9 ± 125.4
Monocrotaline	36.8 ± 5.0	1852.6 ± 280.8	1251.6 ± 59.7	358.6 ± 54.4	108.6 ± 20.0

Cellular uptake of anisidine and monocrotaline via OCTs and MATE transporters

As shown in Figs. 5A and 5B, the intracellular anisidine in OCT1-HEK293, OCT3-HEK293, MATE1-HEK293 and MATE2-K-HEK293 cells showed less than 2.5 folds increase than in mock cells. The inhibitor MPP^+ (for OCTs) or cimetidine (for MATEs) was not able to inhibit that increased uptake. Less than 2.5 folds increase of accumulated compounds in transporter-overexpressing cells than in mock cells was defined as lack of transport in this study, so far no agreement has been reached by International Transporter Consortium regarding this threshold^[18]. In contrast, the uptake of anisidine was increased by 6-fold at $1 \mu\text{mol}\cdot\text{L}^{-1}$ and by 5-fold at $5 \mu\text{mol}\cdot\text{L}^{-1}$ in OCT2-overexpressing cells when compared with mock cells. Uptake of anisidine mediated by OCT2 followed Michaelis-Menten kinetics with K_m of $13.3 \pm 2.6 \mu\text{mol}\cdot\text{L}^{-1}$ and V_{max} of $286.8 \pm 53.6 \text{ pmol}/\text{mg protein}/\text{min}$

(Fig. 5C). Taken together, these results indicated that anisidine was a substrate of OCT2.

The monocrotaline uptake at $5 \mu\text{mol}\cdot\text{L}^{-1}$ in OCT1-HEK293 and OCT2-HEK293 cells was 3 times higher than in mock cells (Fig. 6A). Indeed this uptake mediated by OCT1 or OCT2 followed the Michaelis-Menten equation (Figs. 6C and 6D). Monocrotaline showed a lower affinity to OCT1 than OCT2 with respective K_m values of 109.1 ± 17.8 and $64.7 \pm 14.8 \mu\text{mol}\cdot\text{L}^{-1}$. However, 3-fold higher transport capacity was observed on OCT1 ($V_{\text{max}} = 576.5 \pm 87.5 \text{ pmol}/\text{mg protein}/\text{min}$) than OCT2 ($V_{\text{max}} = 180.7 \pm 22.0 \text{ pmol}/\text{mg protein}/\text{min}$) (Table 2 and Figs. 6C, 6D). On the other hand, OCT3, MATE1 and MATE2-K showed a lack of transport of monocrotaline and the inhibitor MPP^+ (for OCTs) or cimetidine (for MATEs) were not able to inhibit its increased uptake (Figs. 6A and 6B). These results indicated that monocrotaline was a substrate of OCT1 and OCT2, but not of OCT3 and MATEs.



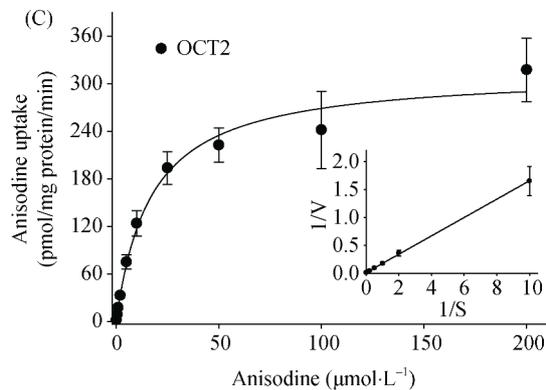


Fig. 5 Anisidine as a substrate of OCTs and MATE transporters. The transporter-overexpressing HEK293 cells and mock cells were incubated with 1, 5 $\mu\text{mol}\cdot\text{L}^{-1}$ anisidine and 5 $\mu\text{mol}\cdot\text{L}^{-1}$ anisidine with the presence of inhibitor [1 $\text{mmol}\cdot\text{L}^{-1}$ MPP⁺ for OCTs (A) and 1 $\text{mmol}\cdot\text{L}^{-1}$ carnitine for MATEs (B)]. Results are shown as the percentage of uptake in mock cells with 1 $\mu\text{mol}\cdot\text{L}^{-1}$ anisidine. Less than 2.5 folds increase than mock cells was defined as lack of transport in this study. Concentration dependent uptake of anisidine mediated by OCT2 (C), the amount of anisidine in mock cells was subtracted from transporter-overexpressing cells. All data are represented as means \pm SEM ($n = 3$)

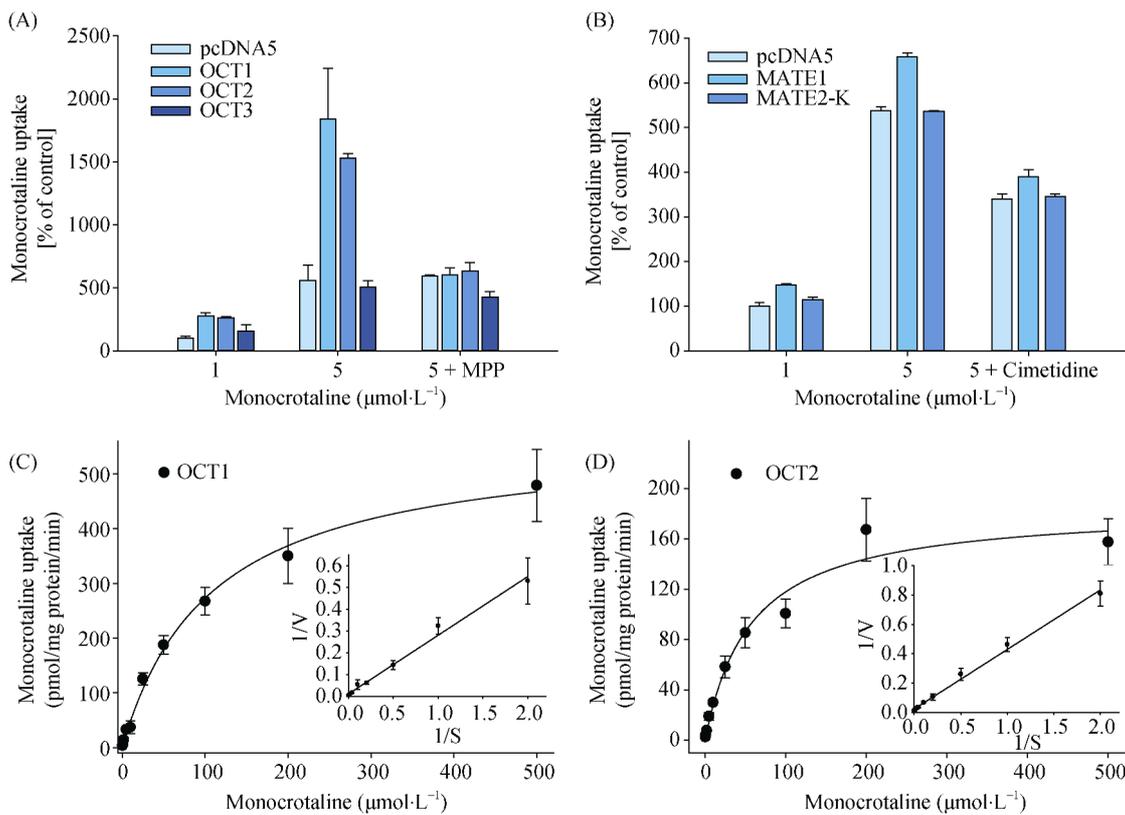


Fig. 6 Monocrotaline as a substrate of OCTs and MATE transporters. The transporter-overexpressing HEK293 cells and mock cells were incubated with 1, 5 $\mu\text{mol}\cdot\text{L}^{-1}$ monocrotaline and 5 $\mu\text{mol}\cdot\text{L}^{-1}$ monocrotaline with the presence of inhibitor [1 $\text{mmol}\cdot\text{L}^{-1}$ MPP⁺ for OCTs (A) and 1 $\text{mmol}\cdot\text{L}^{-1}$ carnitine for MATEs (B)]. Results are shown as the percentage of uptake in mock cells with 1 $\mu\text{mol}\cdot\text{L}^{-1}$ monocrotaline. Less than 2.5 folds increase than mock cells was defined as lack of transport in this study. Concentration dependent uptake of monocrotaline mediated by OCT1 (C) and OCT2 (D). Amount of monocrotaline in the mock cells was subtracted from transporter-overexpressing cells. All data are represented as means \pm SEM ($n = 3$)

Discussion

The consumption of herbal medicines and herbal food

products is steadily increasing. Over the past two decades, consumption rate of herbal medicine in western countries especially in Germany and France has been sharply increased [19].

Furthermore, people in developing countries commonly use herbal medicines [20]. In China, some traditional Chinese medicines were used as substitutes for conventional western medicines due to their low prices [21–22]. However, potential

drug-drug (DDI) and herb-drug interactions (HDI) may occur that mediated by enzymes and transporters. Furthermore pharmacogenomics of those enzymes and transporters may affect the interactions [23].

Table 2 Transport kinetic parameters of anisodine and monocrotaline mediated by organic cation transporters

	$K_m/(\mu\text{mol}\cdot\text{L}^{-1})$	$V_{\text{max}}/(\text{pmol}/\text{mg protein}/\text{min})$	$CL_{\text{int}}(V_{\text{max}}/K_m)/(\mu\text{L}/\text{mg protein}/\text{min})$
Anisodine			
OCT2	13.3 ± 2.6	286.8 ± 53.6	21.6 ± 1.5
Monocrotaline			
OCT1	109.1 ± 17.8	576.5 ± 87.5	5.4 ± 0.5
OCT2	64.7 ± 14.8	180.7 ± 22.0	2.9 ± 0.3

Anisodine has been applied in clinic for anti-circulatory shock since 1970s [24]. Less is known about its pharmacokinetic behavior. Using *in vitro* and *in vivo* models, 20 metabolites of anisodine were identified [3–4]. However, its transport mechanism is fully unknown. On one hand, the PAMPA results indicated that anisodine obtained relatively high permeability [$P_e = (2.9 \pm 0.3) \times 10^{-6} \text{ cm}\cdot\text{s}^{-1}$]. This may explain its high bioavailability and high concentration in brain [25–26]. On the other hand, besides its passive diffusion, the transport results suggest that OCT2 can also mediate anisodine's transport. As anisodine was primarily eliminated by kidney [4, 26], OCT2 may exert an important influence on its renal elimination. Potential DDI and polymorphisms involving OCT2 may affect pharmacokinetics of anisodine, which need to be investigated by further studies.

Lots of herbal elements are toxic although people usually use them with less caution [27]. In recent years, the attentions on herb-drug interaction have been on the rise [28]. Different level requirements have been announced by different countries concerning individual pyrrolizidine alkaloids in food or feed [29]. The hepatotoxin monocrotaline may be ingested and enter systemic circulation as contaminates in foods [30]. This may lead to liver damage, pulmonary hypertension in infants or even some chronic diseases such as cancers [31–34]. Metabolites of monocrotaline can interact with DNA and Protein-Lys-NH₂, which is the main reason for toxicity [5, 35]. It has been reported that OCT1 mediates the first step of monocrotaline transport into hepatocytes [13]. Here we confirmed that monocrotaline is a substrate of OCT1 with a much higher ($K_m = 109 \mu\text{mol}\cdot\text{L}^{-1}$) than previously reported ($K_m = 25 \mu\text{mol}\cdot\text{L}^{-1}$) [13]. Our data also demonstrated that MATE1 cannot transport monocrotaline. To the best of our knowledge there is no data showing that monocrotaline is a substrate of the other canalicular efflux pumps like MDR1 or BCRP. This suggests that a combination of effective influx transport via OCT1 and the lack of efflux transport could lead to increased monocrotaline concentrations in the hepatocytes and increase toxicity. We also showed that monocrotaline is a substrate of OCT2, further investigation need to be done to explore if the transport *via* OCT2 was related to the nephrotoxic effects of monocrotaline [36–38].

In summary, this study investigated interaction of anisodine and monocrotaline with organic cation transporters. It is the first time to suggest that OCT2 may be the most important organic cation transporter for anisodine's renal excretion. The data indicated that OCT1 to be a factor in the hepatotoxicity and OCT2 to play a role in nephrotoxicity of monocrotaline. Anisodine and monocrotaline showed various inhibitory potency to organic cation transporters. Monocrotaline is a substrate of both OCT1 and OCT2. Transporter polymorphisms may influence on pharmacokinetics of anisodine and monocrotaline. However its clinical relevance should be further evaluated.

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