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Potent inhibition of human organic cation transporter 2 (hOCT2) by β-carboline alkaloids

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Abstract

1. Beta-carbolines are indole alkaloids with a wide range of pharmacological and toxicological activities. Beta-carbolines are structurally related to the neurotoxin 1-methyl-4-phenylpyridinium (MPP+), a known substrate of organic cation transporters (OCTs). The goal of this study is to determine the interaction of β-carbolines with human OCT1, 2, and 3 (SLC22A1-3).

2. Dose-dependent inhibition studies were performed for five commercially available β-carbolines using a fluorescent substrate assay in HEK293 cells stably expressing hOCT1-3. The substrate potential was evaluated by uptake assays and the impact of active transport on cellular toxicity examined.

3. All tested β-carbolines potently inhibited hOCT2 with IC_{50} values in the sub- or low micromolar range. Harmaline is the most potent hOCT2 inhibitor (IC_{50}=0.50 ± 0.08 μM). hOCT1 and hOCT3 are less sensitive to β-carboline inhibition. Harmaline, norharmanium, and 2,9-dimethyl-4,9-dihydro-3*H*-β-carbolinium accumulated 2- to 7-fold higher in cells expressing hOCT1-3. HEK293 cells expressing hOCT1-3 were 6.5- to 13-fold more sensitive to harmane and norharmanium toxicity.

4. Our data support a significant role of hOCT1-3 in tissue uptake and disposition of β-carbolines. Importantly, the potent inhibition of hOCT2 by β-carbolines also raises the concern of potential drug interactions between naturally occurring bioactive alkaloids and drugs eliminated by hOCT2.

Keywords

β-Carbolines; organic cation transporters; hOCT2; inhibitor; drug interactions

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Introduction

Many drugs and toxins are hydrophilic organic cations that rely on membrane transporters to cross biological membranes. The polyspecific organic cation transporters 1–3 (OCT1-3) in the solute carrier 22 (SLC22) family play important roles in systemic elimination and tissue-specific disposition of organic cations including many clinically used drugs (Giacomini et al., 2010; Koepsell et al., 2007). OCT1-3 (SLC22A1-3) possess largely overlapping substrate and inhibitor specificities and are differentially expressed in various tissues. In humans, OCT1 is abundantly expressed in the liver, and represents a major transporter for hepatic uptake of cationic drugs such as metformin (Koepsell, 2013). OCT2, on the other hand, is predominantly expressed in the kidney and plays a key role in renal secretion of organic cations (Koepsell, 2013; Yin et al., 2015). OCT3 is broadly distributed in many tissues (brain, heart, placenta, skeletal muscle, liver, kidney etc.) and has been suggested to play a role in tissue-specific uptake of both endogenous biogenic amines as well as xenobiotic organic cations (Chen et al., 2010; Duan & Wang, 2010). Among the OCTs, OCT2 is of particular relevance to pharmacokinetics and drug–drug interactions (DDIs). Localized on the basolateral membrane of renal proximal tubule cells, OCT2 actively transports organic cations from blood into renal epithelial cells, where they can be further effluxed into the urine by the apically localized multidrug and toxin extrusion protein 1 and 2-K (MATE1 and MATE2-K) (Giacomini et al., 2010; Hillgren et al., 2013). OCT2 is thought to be involved in many clinically relevant drug–drug interactions (DDIs) (Li et al., 2006; Morrissey et al., 2013), and is one of the seven drug transporters that are recommended by FDA and International Transporter Consortium for consideration in drug development based on their demonstrated relevance in clinical pharmacokinetics and drug interactions (Giacomini et al., 2010; Zhang et al., 2010).

Beta-carbolines, characterized by a core indole structure fused with a pyridine ring, are a class of naturally occurring indole alkaloids found in plants and animal tissues. Originally identified from the plant *Peganum harmala* (Syria rue), these alkaloids are widespread in our environment and diets and may also be produced endogenously (Pfau & Skog, 2004; Robinson et al., 2003). They can be found in well-cooked meats and fish, coffee, chocolate, alcoholic beverages, tobacco smoke, as well as in herbal products used in traditional medicine or for recreational purpose (Herraiz et al., 2010; Herraiz, 2000a,b, 2004; Nussberger et al., 1987; Totsuka et al., 1999). In humans, there are many potential sources of β-carboline exposure including foods, beverages, and cigarette smoking (Herraiz, 2000a,b, 2004; Totsuka et al., 1999). They have been detected in human body fluids (blood, urine, cerebrospinal fluid) and tissues including brain, liver and kidney (Airaksinen & Kari, 1981; Louis et al., 2010; Matsubara et al., 1993, 1995). Beta-carbolines have a broad spectrum of biological, pharmacological and toxicological activities, including antitumor, antimicrobial, anti-inflammatory, cardiovascular, neuroactive, psychoactive or neurotoxic actions (Du et al., 1997; Glennon et al., 2000; Gockler et al., 2009; Herraiz et al., 2010). Of particular note, the 2N-methylated β-carboline compounds (i.e. β-carbolinium cations), which can be produced through endogenous N-methyltransferase activity, are structurally similar to the neurotoxin MPP+(Storch et al., 2004). Several β-carbolinium cations have been suggested as

environmental neurotoxins underlying idiopathic Parkinson's disease (Gearhart et al., 2002; Hamann et al., 2006; Yang et al., 2008).

Many β-carbolines are extensively metabolized *in vivo* whereas others are significantly excreted into urine (Fekkes & Bode, 1993; Fekkes et al., 2001; Riba et al., 2003, 2012). While the bioactivation, pharmacological and toxicological action of various β-carbolines have long been areas of active research, much less is known regarding their disposition and potential interactions with drug metabolizing enzymes and transporters (Fekkes & Bode, 1993; Fekkes et al., 2001; Riba et al., 2012). Several β-carbolines undergo *O*-demethylation mediated by the cytochrome P450 enzymes, especially CYP2D6 (Herraiz et al., 2013; Jiang et al., 2013; Wu et al., 2009). A recent study also suggested significant inhibition of CYP3A4 (Zhao et al., 2011). These studies have raised the concern of possible pharmacokinetic interactions between beta-carboline-rich natural products and CYP substrate drugs (Jiang et al., 2013; Yu, 2008; Zhao et al., 2011). Currently, little is known about the effect of β-carbolines on renal or hepatic drug transporters. Beta-carbolines are structurally related to MPP+, a known substrate of the OCTs (Koepsell et al., 2007). Several β-carbolines have been shown to be transported by the dopamine transporter (DAT) (Storch et al., 2004) and the plasma membrane monoamine transporter (PMAT) (Ho et al., 2011), which shares a substantial overlap of substrates and inhibitors with the OCTs (Duan & Wang, 2010; Engel & Wang, 2005; Koepsell et al., 2007). Hence, we hypothesized that β-carbolines are inhibitors and/or substrates of the OCTs. Given their increasing prominence as targets for clinical DDIs, we analyzed the interaction of five commercially available β-carbolines with human OCT1-3 using Flp-in HEK293 cells stably expressing these transporters. The transportability of β-carbolines by OCT1-3 was assessed by uptakes assays followed by LC-MS/MS quantification; and the impact of transport on β-carboline cytotoxicity was examined by MTT assay.

Materials and methods

Materials

Harmaline (4,9-dihydro-7-methoxy-1-methyl-3*H*-pyrido[3,4-*b*] indole), harmine (7-methoxy-1-methyl-9*H*-pyrido[3,4-*b*] indole), harmane methosulfate (1-methyl-9*H*-pyrido[3,4-b] indole, methosulfate), norharmanium methiodide (2-methyl-9*H*-β-carboline-2-ium, iodide), 2,9-dimethyl-4,9-dihydro-3*H*β-carbolin-2-ium, iodide], and MTT (thiazolyl blue tetrazolium, bromide) were obtained from Sigma-Aldrich (St. Louis, MO). [^{3}H]MPP +(85Ci/mmol) was purchased from American Radiolabelled Chemicals (St. Louis, MO). ASP+(4-(4-(diethylamino) styryl)-N-methylpyridinium, iodide) and trypan blue were purchased from Life Technologies, Inc. (Carlsbad, CA). Cell culture media and reagents were from Life Technologies, Inc. LC-MS/MS grade acetonitrile, water, and formic acid were purchased from Fisher Scientific (Waltham, MA).

Cell culture

Flp-in HEK293 cell lines stably expressing hOCT1, hOCT2 and hOCT3 at isogenic locations were previously generated in our laboratory (Duan & Wang, 2010; Duan et al., 2015). A HEK293 cell line stably transfected with the empty pcDNA5/FRT vector served as

control. The cells were cultured in D-MEM (high glucose) media supplemented with 10% FBS, 2mM L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin and 150 μg/ml hygromycin B in a 37°C humidified incubator with 5% CO_2. For better attachment of cells, all cell culture plastic surfaces were coated with 0.01% poly L-ornithine (MW 30 000–70 000) in phosphate buffered saline solution before plating.

β-Carboline inhibition studies

Interaction between β-carbolines and hOCT1-3 were assessed with a fluorescent ASP+-based uptake assay as we described previously (Duan et al., 2015). The assay uses ASP+as substrate and trypan blue as extracellular fluorescence-quenching dye. The uptake cocktail consists of 2 μM ASP+and 10 μM trypan blue in uptake buffer (1× Hank's Balanced Salt Solution and 20mM HEPES, pH 7.4). Cells were seeded at a density of 100 000 cells/well in 96-well plates and grown overnight. Uptake assays was performed at 37 °C. All reagents were pre-warmed to 37 °C. Immediately before the assay, cells were washed once and 100 μl of uptake buffer containing β-carbolines at graded concentrations was transferred to the wells. Uptake was initiated by adding 100 μl of ASP+uptake cocktail. Relative fluorescence (RFU) was recorded for each well immediately after adding ASP+(time 0) and at the end of the uptake experiment (5 min). Specific uptake was calculated by subtracting the fluorescence readings at time 0 from the end point (RFU_{end}–RFU_{time0}). Specific uptake in the absence of any inhibitors was normalized as 100%. Florescence measurements were done from a bottom-read position in a Perkin-Elmer Wallac 1420 Multilabel Counter capable of precise temperate control and kinetic measurements. For ASP+, the excitation/emission wavelengths are 475 nm/609 nm. Excitation and emission filters were configured within 10 nm of these wavelengths. Uptake experiments were repeated independently three times.

β-Carbolines uptake assay

Uptake assays were performed as previously described with modification for the analysis of β-carbolines by liquid chromatography tandem mass spectrometry analysis (Duan & Wang, 2010; Duan et al., 2015; Yin et al., 2015). Cells were seeded in 96-well plates at 100 000 cells/well and grown overnight. Uptake assays were performed at 37 °C. All reagents were pre-warmed to 37 °C. Immediately before the assay, cells were washed twice with HBSS and allowed to acclimate for 10 minutes at 37 °C. Media was emptied and incubations initiated by the addition of HBSS containing substrate (1 μM). Uptake was terminated at 30 minutes by removing media and washing three times with ice cold HBSS. Cells were lysed by addition of acetonitrile containing an internal standard and β-carbolines were quantified by LC-MS/MS. For [^{3}H]MPP+uptake, cells were lysed with 1 M NaOH and neutralized with 1 M HCl; and intracellular MPP+was quantified by liquid scintillation counting (PerkinElmer, Tri-Carb B3110TR, Waltham, MA). Uptake was normalized to total protein measured using a BCA Protein Assay Kit (Pierce Chemical, Rockford, IL).

Quantification of β-carbolines by LC-MS/MS

Beta-carbolines were quantified in cell lysate by LC-MS/MS using published methods with modification (Meyer et al., 2014; Oliveira et al., 2012; Richling et al., 1996). Glyburide was used as the internal standard. An AB Sciex 4000 QTRAP Mass Spectrometer (AB Sciex,

Framingham, MA) coupled to an Acquity UPLC system (Waters corporation, Milford, MA) was operated in electrospray ionization (ESI) positive mode. An Agilent Zorbax Eclipse plus C18 column (1.8 um, 2.1 × 50 mm) was used with a mobile phase consisting of 0.1% v/v formic acid in water (A) 0.1% formic acid in acetonitrile (B). A box gradient with 0.45 ml/min flow starting with 10% B until 0.1 minutes and 90% B from 0.1 to 1.5 minutes followed by 0.5 minutes at 10% B eluted β-carbolines at 0.9 minutes. Mass transitions (*m/z*) were 213.2→170.1 for harmine, 215.1→176.1 for harmaline, 183.2→114.9 for harmane, 183.2→114.9 for norharmanium, 185.1→139.2 for 2,9-dimethyl-4,9-dihydro-3*H*-β-carbolin-2-ium, and 494.1→369 for the internal standard glyburide. Instrument control and data processing were performed using Analyst software version 1.6.2. Accuracy was within 15% (20% for the lower limit of quantification).

β-Carboline toxicity assay

The MTT assay was used to determine cytotoxicity of β-carbolines toward pcDNA5 and hOCT1-3 stably transfected HEK293 cells as we described previously (Ho et al., 2011). Cells were seeded in 96-well plates at a density of 14 000 cells/well and grown overnight to reach ~20% confluency. The cells were then incubated with fresh media containing β-carbolines at graded concentrations for an additional 72 h. The cells were then washed once with warm Dulbecco's phosphate-buffered saline, and 0.1 ml/well of MTT working solution (0.5 mg/ml MTT in 1X Hank's Balanced Salt Solution with 20mM HEPES) was added into each well. After incubation at 37 °C for 1 h, the MTT solution was aspirated away, and 0.1 ml/well acidic isopropanol (0.04 M HCl in absolute isopropanol) was added. The plate was mixed on a rotating platform vigorously for 15 minutes to completely dissolve MTT formazan precipitates (Ho et al., 2011). Absorbance was measured at a wavelength of 570 nm to determine cell viability. As we observed a systematic increase (10–25%) in optical intensity in cells treated with all β-carbolines at low and nontoxic concentrations, which is probably due to additional optical absorbance by residual β-carbolines, the survival rate in cells treated with the lowest drug concentration was set to 100% and used as a control to normalize cell viability across the entire concentration range.

Data analysis

The half maximum inhibition concentration (IC_{50}) and the half maximum toxic concentration (TC_{50}) were determined by non-linear regression fit of uptake or cell viability data with the log(inhibitor) versus response–variable slope (four parameters) model in GraphPad Prism® v5.0. The equation is: $Y=Bottom+(Top-Bottom)/(1+10^{((LogIC50-X)*H)})$, where Y is the % specific uptake or cell survival, Bottom is the residual uptake or survival, Top is the maximal uptake or survival, X is β-carboline concentration and H is the hill slope. For cell viability, TC_{50} replaces IC_{50}. The constraints were set as Bottom ≥0. Data are expressed as Mean±SD of independent experiments and repeated at least three times. The *p* values were determined by Student's *t*-test in GraphPad Prism® 5.0. (La Jolla, CA).

Results

β-Carbolines inhibit hOCT1-3 mediated ASP + uptake

The ASP+uptake assay is a rapid, non-radioactive, fluorescent substrate-based method that has been previously used to characterize compound interaction with the OCTs (Ciarimboli et al., 2005; Duan et al., 2015; Kido et al., 2011; Mason et al., 2005). We previously demonstrated that the IC_{50} values obtained by the fluorescence assay correlate well with IC_{50} values determined by traditional radiotracer assays (Duan et al., 2015). Here, we applied the assay to study hOCT interaction with commercially available β-carbolines. The structures of the five tested β-carbolines are shown in Figure 1. These include two methoxy harmala alkaloids (harmine, harmaline), a non-methoxy substituted analog (harmane), and two β-carbolinium cations (norharmanium, 2,9-dimethyl-4,9-dihydro-3*H*-β-carbolinium). Concentration-dependent inhibition of ASP+uptake was observed for all five β-carbolines towards hOCT1-3 (Figure 2). The fitted IC_{50} values are summarized in Table 1. No apparent relationship was observed between the molecular structures of β-carbolines (Figure 1) and their inhibition potencies (Table 1) for a specific transporter. The 2N-methylated β-carbolinium cations (norharmanium, 2,9-dimethyl-4,9-dihydro-3*H*-β-carbolinium) showed comparable potencies to the non-permanently charged β-carbolines (harmine, harmaline, harmane). The 3,4-carbon saturation on the pyridine ring (harmaline versus harmine), and the methoxyl substitution on position 7 of the indole ring (harmine versus harmane) also did not have a major effect on the β-carboline interaction with the OCTs.

For all five β-carbolines, hOCT2 showed much greater sensitivity with IC_{50} values in sub- or low micromolar range. Harmaline, a psychoactive alkaloid present in *Peganum harmala* as well as in the hallucinogenic drink ayahuasca, showed the most potent inhibition towards hOCT2 (IC_{50}=0.479±0.077 μM). In contrast, hOCT1 was 1–2 order less sensitive to β-carboline inhibition. hOCT3 showed an intermediate sensitivity. These data suggest that β-carbolines are potent inhibitors of hOCT2, a major transporter involved in the elimination of cationic drugs in the kidney.

Permanently charged β-carbolines are substrates of hOCT1-3

Direct determination of β-carbolines (1 μM) hOCT substrate potential was performed by measuring accumulation in vector and hOCT1-3-transfected cells by LC-MS/MS quantification (Figure 3). The positive control MPP+accumulated 4- to 10-fold relative to vector cells in hOCT1-3 transfected cells at 30 minutes. Substantially higher accumulation (5- to 7-fold) was observed in hOCT1-3 transfected cells with norharmanium and 2,9-dimthyl-4,9-dihydro-3H-β-corbolin-2-ium, suggesting these two permanently charged cations are substrates of all three hOCT isoforms. Harmaline demonstrated ~2-fold higher accumulation in hOCT1 and hOCT3. Harmine and harmane did not show significantly higher accumulation in hOCT1-3 transfected cells as compared with vector controls at 1 μM.

Impact of hOCT1-3 expression on β-carboline cytotoxicity

Beta-carbolines are structurally related to MPP+, which exerts mitochondrial toxicity by inhibiting complex I of the mitochondrial respiratory chain. To test whether expression of hOCTs would impact intracellular toxicity of β-carbolines, we examined if the apparent

toxicity of these compounds to HEK293 cells are altered using an MTT assay. The dose-dependent toxic response curves of the five β-carbolines in pcDNA5- (control) and hOCT1-, hOCT2-, and hOCT3-transfected cell lines are shown in Figure 4. The fitted TC_{50} values are summarized in Table 2. MPP+and all five tested β-carbolines showed innate toxicity in control HEK293 cells (Table 2). As expected, expression of hOCT1, hOCT2, and hOCT3 increased HEK293 cell sensitivity to MPP+by 16.4, 8.9 and 10.9 folds, respectively (Table 2). Similar to MPP+, harmane and norharmanium exhibited greatly increased toxicity in all three transporter-transfected cells. These data suggest that transport mediated accumulation of norharmanium by hOCT1, 2 and 3 in HEK293 cells increased its apparent potency. Compared to vector-transfected HEK293 cells, expression of hOCT1-3 had no significant effect on cellular response to harmine, harmaline, and 2,9-dimethyl-4,9-dihydro-3*H*-β-carbolin-2-ium.

Discussion

Naturally occurring in plants, foods, and animal tissues, β-carboline alkaloids have been found in human tissues and body fluids. They are a major form of alkaloid in the seeds of *Peganum harmala* (Syria rue), which has been used in traditional medicine and for recreational purposes (Herraiz et al., 2010; Nussberger et al., 1987). Some β-carbolines, such as harmine and harmaline, are also the major psychotropic ingredients in Ayahuasca, a religious and recreational tea that was originally used by indigenous Amazonia tribes but has also spread to the United States and elsewhere (McKenna, 2004; Riba et al., 2003). The psychotropic effect of harmine and harmaline in Ayahuasca is largely due to their potent inhibition of monoamine oxidase A (MAO-A), which metabolically inactivates other psychedelic indolealkylamines co-present in Ayahuasca (Kim et al., 1997; Rommelspacher et al., 1994; Smith et al., 2013). Recent studies have revealed interactions between β-carbolines and CYP2D6 (Herraiz et al., 2013; Jiang et al., 2013; Wu et al., 2009), raising the concern of possible pharmacokinetic interactions (Jiang et al., 2013; Yu, 2008; Zhao et al., 2011). Because β-carbolines are structurally related to the OCT substrate MPP+, the goal of this study is to determine if β-carbolines are inhibitors and/or substrates for human OCT1-3.

In the human kidney, the basolateral hOCT2 and apical hMATEs sequentially mediate tubular secretion of many organic cation drugs (Wagner et al., 2016). According to the recent draft FDA guidance (Food and Drug Administration, 2012), OCT2 is one of three drug transporters in the human kidney recommended for evaluation for potential DDIs during drug development. Our results (Table 1 and Figure 2) showed that while hOCT1-3 are all inhibited by β-carbolines, hOCT2 is most sensitive to these indole alkaloids with IC_{50} values in sub- to low micromolar range. Although several synthetic compounds, such as decynium 22, tetrapentylammonium, and N-butyl-N-methylpyrrolidinium, are identified as potent hOCT2 inhibitors with reported IC_{50} or K_i values in the same range as the β-carbolines (Cheng et al., 2011; Gorboulev et al., 1997), their use is limited to in vitro assays and animal studies. In contrast, most clinically used hOCT2 inhibitors are much less potent, with affinities in mid-to high micromolar range (Morrissey et al., 2013; Motohashi & Inui, 2013). For example, cimetidine, the classic blocker of OCT2, has a reported K_i of 95–146 μM for hOCT2 expressed in HEK293 cells (Ito et al., 2012). In contrast, the apically localized MATEs proteins is more sensitive to cimetidine (K_i=1–4 μM). Because clinically

encountered concentrations of cimetidine in plasma is about 2.0–3.6 μM, it has been suggested that inhibition of hMATEs, rather than hOCT2, underlines renal cimetidine–drug interactions (Ito et al., 2012). Here, we show that β-carbolines, which occur both endogenously and exogenously in diets and herbal products, are much more potent *in vivo* inhibitors for hOCT2. The most potent inhibitor is harmaline (IC_{50} ~0.5 μM), a major psychotropic ingredient in the Amazonia tea Ayahuasca. The reported endogenous β-carboline concentrations in human body and fluids are generally in the low nanomolar range (Gearhart et al., 2002; Matsubara et al., 1993; Parker et al., 2004), and therefore will not affect the function of hOCT2. However, after a single ingestion of Ayahuasca in healthy volunteers, plasma β-carbolines, including harmaline, reportedly reached mid to high nanomolar range (Riba et al., 2012), which may lead to significant inhibition of hOCT2. This interaction may be further aggregated by multiple Ayahuasca ingestions and/or in CYP2D6 poor metabolizers since harmaline is metabolized primarily via *O*-demethylation by CYP2D6. Thus, our study suggests potential herb- or food–drug interactions at the site of hOCT2 as a result of potent inhibition of the transporter by β-carbolines in diets and natural products.

Beta-carbolines exhibit a broad spectrum of biological and pharmacological activities due to their binding to benzodiazepine, imidazoline, and serotonin receptors as well as reversible inhibition of intracellular MAO (Glennon et al., 2000; Herraiz et al., 2010; Parker et al., 2004; Robinson et al., 2003). Many β-carbolines are readily absorbed and distributed in body tissues and brain (Louis et al., 2010; Matsubara et al., 1993, 1995). β-carbolines (e.g. harmine, harmaline, norharman) are extensively metabolized by the liver while others are excreted into urine. Our finding that norharmanium, 2,9-dimethyl-4,9-dihydro-3*H*-β-carbolin-2-ium, and harmaline are accepted as substrates by hOCT1-3 suggests that hOCTs are likely to play a role in the disposition and action of these β-carbolines (Figure 3). Transport-mediated disposition may be particularly important for the permanently charged norharmanium and 2,9-dimethyl-4,9-dihydro-3*H*-β-carbolin-2-ium which are less membrane permeable. For example, hOCT1, which is expressed on the sinusoidal membrane of hepatocytes (Koepsell et al., 2007; Nies et al., 2011), may mediate β-carboline uptake into the liver, where they can be further metabolized by CYP2D6 or other liver enzymes. The kidney-specific hOCT2, on the other hand, may play a role in renal excretion of certain β-carbolines. hOCT3, which is broadly expressed in many neuronal tissues and astrocytes (Cui et al., 2009), could play a role in brain distribution and neurotoxicity of β-carbolines. The non-charged β-carbolines (harmine, harmaline, harmane) had much higher passive diffusion and showed minimal enhanced accumulation in hOCT1-3 transfected cells. Therefore, hOCTs may play a minor role in the disposition of these non-charged β-carbolines *in vivo*.

We found that expression of hOCT1-3 greatly sensitized HEK293 cells to MPP+, norharmanium and harmane cytotoxicity (Figure 4). Norharmanium and 2,9-dimethyl-4,9-dihydro-3*H*-β-carbolin-2-ium were both good substrates of hOCT1-3 but only norharmanium showed increased cytotoxicity. It is important to note that overall toxic response depends on the innate cytotoxicity of these compounds and is also influenced by their passive diffusion across the cell membranes. It is possible that the toxic effect of 2,9-dimethyl- 4,9-dihydro-3*H*-β-carbolin-2-ium towards HEK293 cells involves multiple cellular activation steps and cellular uptake of these compounds is not a rate-limiting step.

Surprisingly, hOCT-expressing cells also showed significantly increased sensitivity to harmane, which is not a substrate of the hOCTs (Figure 3). The reason for this observation is unknown. Previous studies suggest that the 2N-methylated β-carbolinium cations are the active toxic species (Hamann et al., 2006; Storch et al., 2004; Yang et al., 2008). It is possible that harmane itself is not toxic, but undergoes 2N-methylation in HEK293 cells to generate a cytotoxic carbolinium species, which could be a substrate of the hOCTs. Expression of the hOCTs may increase the retention of the toxic species, enhancing its cytotoxic effect in these cells. The disagreement between substrate status and cytotoxicity highlights the importance of directly confirming transporter substrates by uptake assays.

In summary, we show that β-carbolines interact with human OCT1-3 with differential inhibition potencies towards these transporters. We also confirmed that harmaline, norharmanium, and 2,9-dimethyl-4,9-dihydro-3*H*-β-carbolin-2-ium are transportable substrates of hOCT1-3. Together, our data support a role of hOCT1-3 in tissue uptake and disposition of β-carbolines. More importantly, the potent inhibition of hOCT2 by β-carbolines also raises a concern of potential drug interactions between naturally occurring β-carboline alkaloids and drugs that are renally eliminated via hOCT2.

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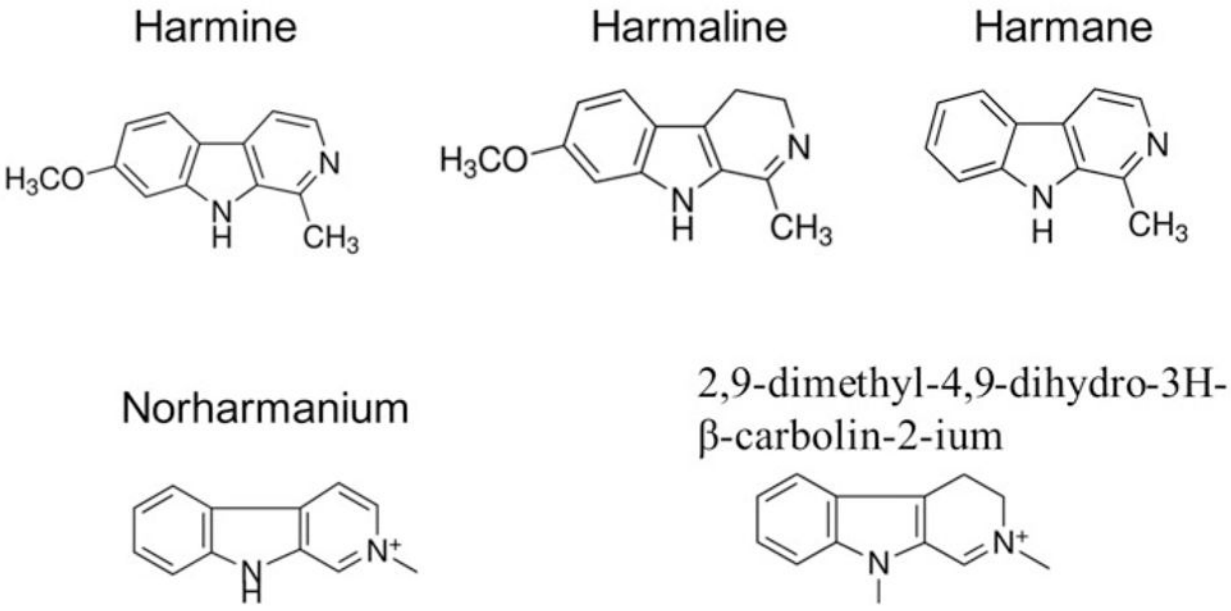

Figure 1.
Molecular structure of β-carbolines used in the current study.

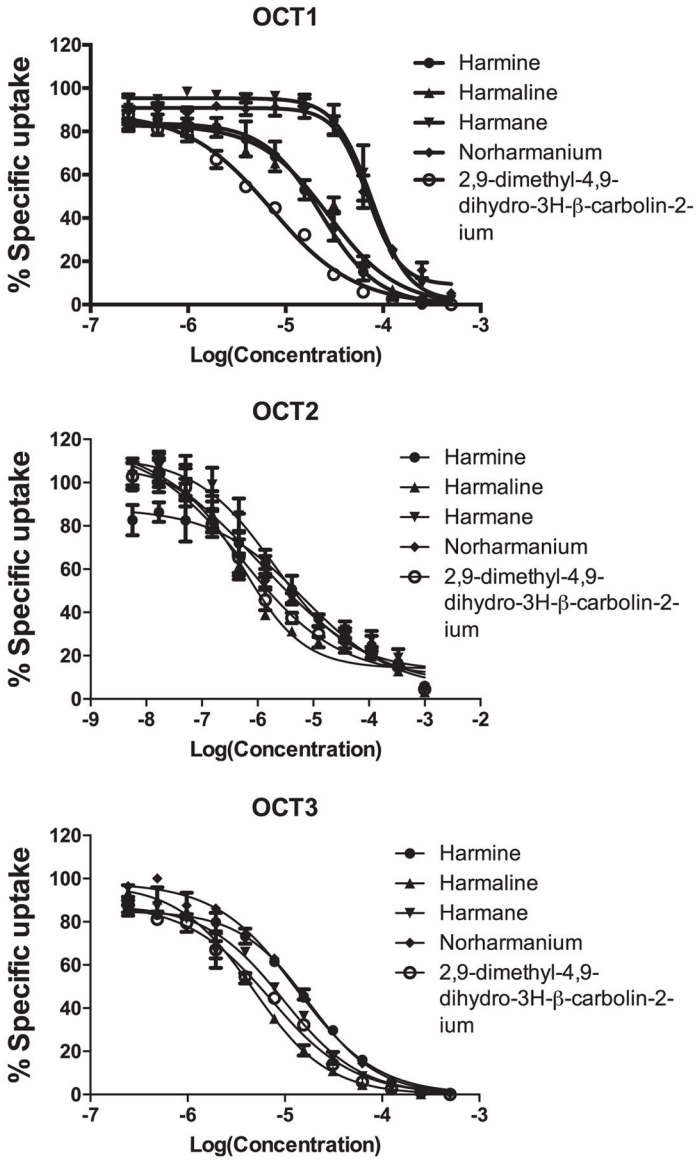

Figure 2.

Interaction of β-carbolines with hOCT1-3. Concentration-dependent inhibition of hOCT1-3 was determined with ASP+-based fluorescence uptake assay. Data represent mean±SD of three independent experiments.

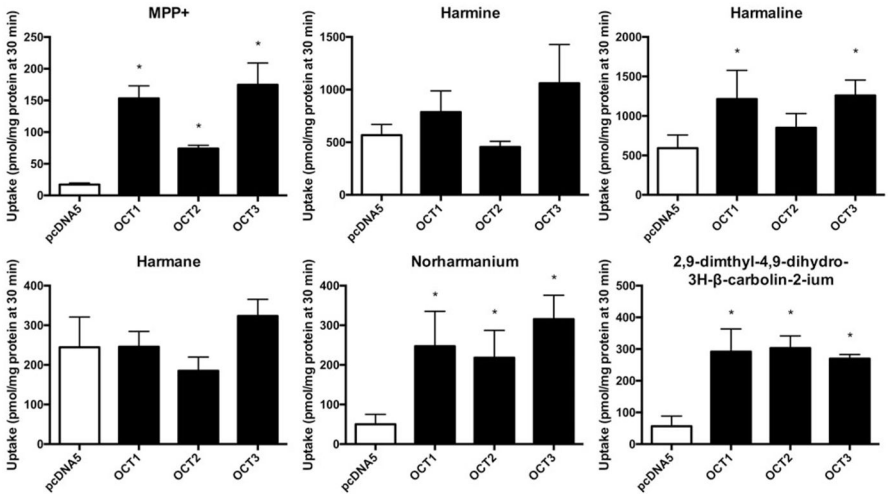

Figure 3.

Uptake of 1 μM MPP+(positive control) and β-carbolines in vector (pcDNA5) or hOCT1-3 stably transfected Flp-in HEK293 cells. Data represent Mean±S.D. of three independent experiments. *Indicates values in transporter-expressing cells significantly different from those in pcDNA5 control cells (p<0.005).

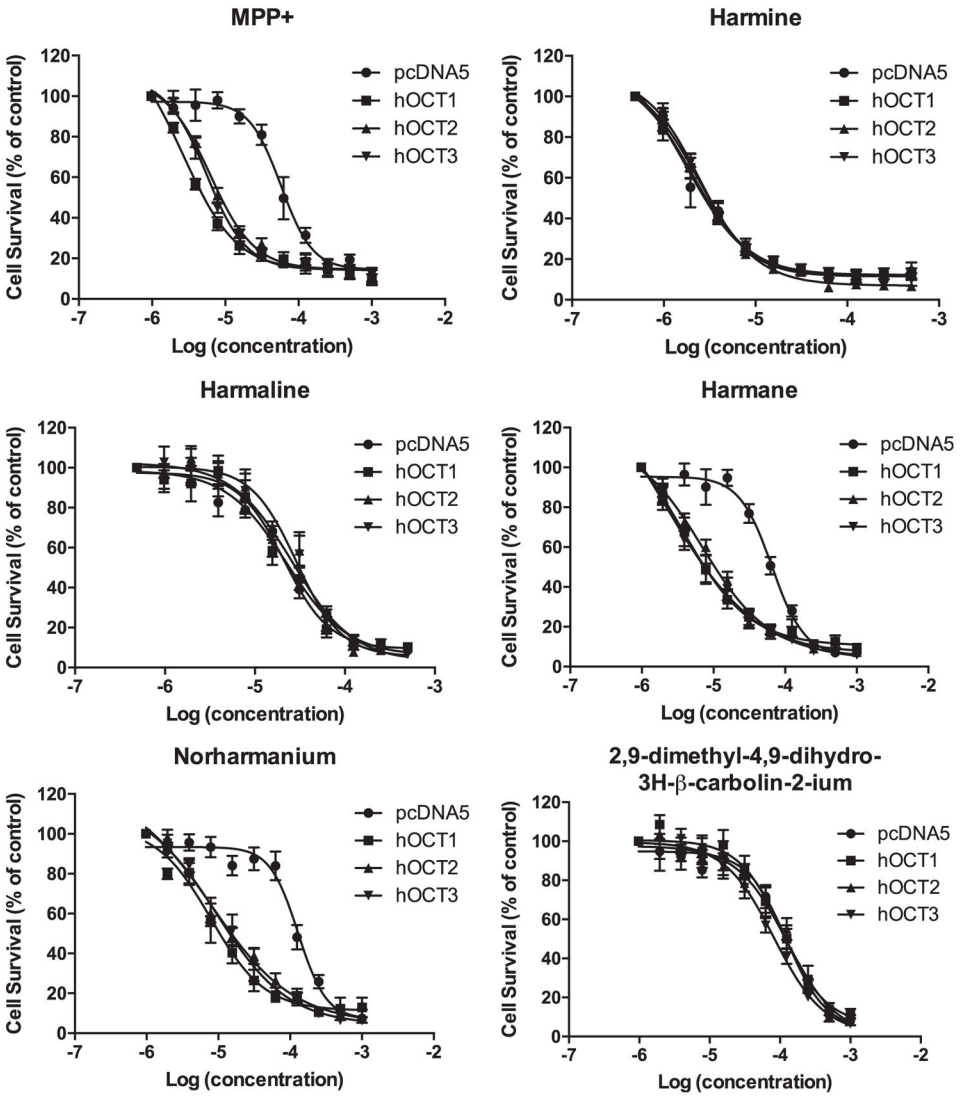

Figure 4.

Concentration-dependent toxicity of MPP+(positive control) and β-carbolines in vector (pcDNA5) or hOCT1-3 stably transfected Flp-in HEK293 cells. Data represent mean±SD of six independent experiments.

Table 1

IC_{50} values of β-carbolines for hOCT1-3 as determined by the ASP+-based uptake assay.

	IC_{50} (μM)		
Compound	**OCT1**	**OCT2**	**OCT3**
Harmine	23.1±2.8	6.14±0.70	17.7±1.0
Harmaline	27.6±3.8	0.497±0.077	4.76±0.46
Harmane	75.5±10.2	2.04±0.35	10.1±0.6
Norharmanium	70.5±6.6	1.05±0.06	13.1±0.1
2,9-Dimethyl-4,9-dihydro-3H-β-carbolin-2-ium	40.4±2.4	0.524±0.088	7.30±0.50

Results represent Mean±SD of three independent experiments.

Table 2

TC_{50} values of β-carboline toxicity towards pcDNA5 (control) and OCT1-3 transfected cells, as determined with the MTT cytotoxicity assay.

	TC_{50} (μM)				Fold increase over pcDNA5		
Compound	**pcDNA5**	**OCT1**	**OCT2**	**OCT3**	**OCT1**	**OCT2**	**OCT3**
MPP+	63.6±12.7	3.88±0.38[a]	7.16±0.75[a]	5.86±0.68[a]	16.4	8.9	10.9
Harmine	2.87±0.47	2.55±0.26	3.01±0.41	2.81±0.23	1.1	0.95	1.0
Harmaline	23.0±3.6	23.0±5.1	35.5±3.8	33.1±8.2	1.0	0.65	0.69
Harmane	66.6±4.6	7.16±1.96[a]	10.3±1.5[a]	8.88±2.76[a]	9.3	6.5	7.5
Norharmanium	119±18	9.05±2.54[a]	13.4±2.4[a]	10.8±1.4[a]	13.1	8.9	11.0
2,9-Dimethyl-4,9-dihydro-3H-β-carbolin-2-ium	127±18	119±33	117±22	80.6±7.2	1.1	1.1	1.6

Data represent mean±S.D. of six independent experiments.

[a]Indicates values in transporter-expressing cells significantly different from those in pcDNA5 control cells ($p<0.005$).