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Effects of Tricyclic Compounds on the Transport of Anti-migraine Triptans through Human Organic Anion Transporting Polypeptide 1A2 (OATP1A2)

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Abstract

OATP1A2 is a membrane drug-transporter expressed at the human blood-brain barrier (BBB) that may potentially mediate penetration of drugs in the brain. Triptans, hydrophilic antimigraine drugs, are substrates of OATP1A2. It is believed that triptans should cross the BBB to reach their site of action. Thus, OATP1A2 can limit brain penetration of triptans and may consequently influence their antimigraine drug action. We have previously demonstrated that compounds composed of a tricyclic ring with a short aliphatic amine chain, such as tricyclic antidepressants and carvedilol, inhibited OATP1A2-mediated rosuvastatin uptake. The main objective of this study was to determine whether triptans transport via OATP1A2 is affected by tricyclic compounds. First, we confirmed that triptans were substrates of OATP1A2 but not OATP2B1 using HEK293 stable cell lines. The tricyclic drugs evaluated were able to inhibit OATP1A2-mediated uptake of triptans. carvedilol was the most potent inhibitor. Potential inhibition was assessed with a range of total plasma concentrations of the drugs. Carvedilol and nortriptyline lowered the uptake of both almotriptan and zolmitriptan whereas clomipramine diminished the uptake of almotriptan only. Our data suggest that these three drugs may limit the penetration of triptans to the brain by modulating OATP1A2 transport at clinically relevant concentrations.

Keywords: Drug transporter; OATP1A2; drug-drug interaction; triptans; blood-brain barrier (BBB)

Introduction

Migraines are an important cause of disability in Canada, affecting 8.3% of the population (2.7 millions) [1]. Triptan drugs are typically used in the treatment of acute migraine attacks. Triptans are selective agonists of the serotonin receptors 5-HT_{1B} and 5-HT_{1D} located on smooth muscle cells of intracranial and extracerebral blood vessels as well as on trigeminal sensory neurons [2-4]. Their mechanisms of action are believed to imply inhibition of activated trigeminal neurons, inhibition of neuropeptides release, interruption of pain transmission, and perhaps selective vasoconstriction of cranial blood vessels [5]. It appears that triptans are required to cross the blood-brain barrier (BBB) to reach their target site in the central nervous system (CNS). However, these drugs are hydrophilic, limiting their penetration through the BBB. Thus, transport mechanisms must exist to facilitate their entrance into the brain. Many membrane drug transporters are expressed at the BBB to limit or facilitate the access of drugs to the brain. Among those involved in drug influx, OATP1A2 and OATP2B1 proteins are expressed on the luminal membrane of the endothelial cells making up the BBB [6-9]. Their physiological roles at the BBB may implicate the distribution of thyroid hormones (triiodothyronine and thyroxine) to the CNS by OATP1A2 and the transport of conjugated neuroactive steroids (pregnenolone sulfate and dehydroepiandrosterone-3-sulfate) to the brain by OATP2B1 [10,11].

OATP1A2 and OATP2B1 transport a wide spectrum of endogenous compounds and xenobiotics while having overlapping substrate selectivity. Recently, a study screened 36 CNS-active drugs for transport through OATP1A2 and has shown that triptans are OATP1A2 substrates [12]. Using triptan structural analogs, a structure-activity relationship was established where an amine residue was essential for transport through OATP1A2 and the uptake rate was the highest for tertiary amine followed by secondary and then primary amines. These findings are interesting as it would suggest that OATP1A2 may facilitate permeation of triptans to the brain.

We have previously demonstrated that the transport of rosuvastatin through OATP1A2 can be inhibited by compounds composed of a tricyclic ring and a short aliphatic amine chain, such as tricyclic antidepressants and carvedilol [13].

The objectives of this study were to:

- Confirm triptans as OATP1A2 substrates in our human embryonic kidney (HEK293)-OATP1A2 stable cell line.
- Determine whether triptans are OATP2B1 substrates using a HEK293-OATP2B1 stable cell line.
- Determine whether compounds composed of a tricyclic ring and a short aliphatic amine chain inhibit the transport of triptans through OATP1A2.
- Determine whether tricyclic compounds can inhibit OATP1A2-mediated uptake of triptans at total plasma concentrations. The consequence of such an interaction in humans would be a diminishment or abolishment in antimigraine efficiency by a limited delivery of triptans into the brain.

Materials and Methods

Reagents

Amitriptyline hydrochloride, carbamazepine, carbazole, chlorpromazine hydrochloride, clomipramine hydrochloride, desipramine hydrochloride, imipramine hydrochloride, naratriptan hydrochloride, nortriptyline hydrochloride, phenothiazine, rizatriptan benzoate, sumatriptan succinate, trimipramine maleate salt, zolmitriptan were purchased from Sigma-Aldrich (St-Louis, MO, USA). Almotriptan hydrochloride, carazolol hydrochloride, carvedilol, doxepin hydrochloride, eletriptan hydrobromide were purchased from Toronto Research Chemicals (Toronto, ON, Canada). All chemicals and solvents were obtained from Sigma-Aldrich, Fisher Scientific (Fair Lawn, NJ, USA) or J.T. Baker (Center Valley, PA, USA).

Cell culture

HEK293-OATP1A2, HEK293-OATP2B1, and HEK293-VC cells were kindly provided by Dr. Markus Keiser and Dr. Werner Siegmund (Department of Clinical Pharmacology, Center of Drug Absorption and Transport, University Medicine Greifswald, Greifswald, Germany). The cells were cultured in minimum essential medium supplemented with 10% fetal bovine serum, 1X nonessential amino acids, and 1X sodium pyruvate at 37°C and 5% CO_2. Cell culture media and supplements were purchased from Multicell Wisent Inc. (St-Jean-Baptiste, QC, Canada); whereas, fetal bovine serum was obtained from HyClone Thermo Scientific (Logan, UT, USA).

Uptake assays and competition assays

Reproducibility of our HEK293-OATP1A2 cell model was assessed with 2-3 different cell batches and comparable K_m values were obtained. The uptake assays were performed as previously described [13]. Briefly, tissue culture plates (6-well or 12-well) were first treated with poly-L-lysine (Sigma-Aldrich, St-Louis, MO, USA) before seeding the HEK293-OATP1A2, HEK293-OATP2B1, and HEK293-VC cells. The number of cells seeded in 6-well and 12-well plates was 1.5×10^6 cells/well and 7.5×10^5 cells/well, respectively. After 24 h, the culture media was replaced with warm transport buffer (142 mM NaCl, 5 mM KCl, 1 mM K_2HPO_4, 1.2 mM $MgSO_4$, 1.5 mM $CaCl_2$, 5 mM glucose, and 12.5 mM HEPES, pH 7.3) and pre-incubated at 37°C for 5 min. Following the pre-incubation period, the cells were incubated with warm transport buffer containing the substrate in the presence or absence of an inhibitor at 37°C. After incubation, the cells were washed twice with phosphate-buffered saline (PBS) containing 10% acetonitrile followed by a final wash with PBS. Time-dependent uptake experiments through OATP1A2 were done in six-well plates by incubating HEK293-OATP1A2 and HEK293-VC cells with drugs at determined K_m, i.e., almotriptan (5 µm), eletriptan (1 µm), naratriptan (20 µm), rizatriptan (43 µm), sumatriptan (94 µm), or zolmitriptan (21 µm). The K_m and V_{max} of the different triptans transport through OATP1A2 was determined by incubating HEK293-OATP1A2 and HEK293-VC cells in six-well plates with almotriptan (0.375-25 µm), eletriptan (0.125-5 µm), naratriptan (0.625-100 µm), rizatriptan (0.75-250 µm), sumatriptan (1.5-500 µm), and zolmitriptan (0.75-250 µm). To determine whether a compound can block OATP1A2-mediated transport of triptans, HEK293-OATP1A2 and HEK293-VC cells were seeded in 12-well plates and co-incubated with almotriptan (15 µm), eletriptan (3 µm), naratriptan (60 µm), rizatriptan (130 µm), sumatriptan (300 µm), or zolmitriptan (65 µm) in the absence or presence of different tricyclic compounds (0.15-150 µM). In the inhibition studies, a concentration of triptan at three times the K_m value was selected in order to saturate the OATP1A2 transporter with the substrate. An incubation time of 2 min was chosen for almotriptan, naratriptan, rizatriptan, sumatriptan, and zolmitriptan; whereas 1 min was chosen for eletriptan. Time-dependent uptake of triptans at clinically relevant concentrations was done in six-well plates by incubating HEK293-OATP1A2 and HEK293-VC cells with almotriptan (50 ng/mL) or zolmitriptan (3 ng/mL). To determine whether clinically relevant concentrations of tricyclic compounds can inhibit OATP1A2-mediated transport of triptans, HEK293-OATP1A2 and HEK293-VC cells were seeded in six-well plates and co-incubated with either almotriptan (50 ng/mL) or zolmitriptan (3 ng/mL) for 1 or 2 min, respectively, in the absence or presence of different tricyclic compounds (10-200 ng/mL).

	Almotriptan	Eletriptan	Naratriptan	Rizatriptan	Sumatriptan	Zolmitriptan
Buffer	10 mM AF pH 3.0	10 mM AF pH 3.0	10 mM AF pH 3.0	10 mM AF pH 3.0	10 mM AF pH 3.0	10 mM AF pH 3.0
Solvent	Methanol	Methanol	Methanol	Methanol	Methanol	Methanol
% Buffer/Solvent	65/35	48/52	68/32	71/29	68/32	80/20
Run time (min)	22	30	20	21	20	24
Flow (ml/min)	0.5	0.5	0.5	0.5	0.5	0.5
Wavelength (nm)	283	272	284	282	283	283
Temperature (°C)	40	50	40	50	40	50
t_R (min)	19	15.7	18.1	9	8.9	21.7
t_R IS (min)	15.2 (IS: naratriptan)	27.5 (IS: doxepin)	8.8 (IS: sumatriptan)	17.9 (IS: naratriptan)	18.5 (IS: naratriptan)	17.8 (IS: rizatriptan)
AF: Ammonium Formate; t_R: Retention Time; IS: Internal Standard						

Table 1: HPLC-UV quantification methods details.

To determine whether triptans are transported by OATP2B1, HEK293-OATP2B1 and HEK293-VC cells were seeded in six-well plates and incubated with almotriptan (5 and 25 μM), eletriptan (1 and 3.75 μM), naratriptan (20 and 60 μM), rizatriptan (40 and 120 μM), sumatriptan (100 and 300 μM), or zolmitriptan (21 and 65 μM). The concentrations chosen for each substrate correspond to its K_m and 3 times K_m value determined in HEK293-OATP1A2 cells. The protein concentration was measured using the Pierce BCA protein assay kit from Thermo Scientific (Rockford, IL, USA). Three wells of each cell line were lyzed with 1% SDS+0.2 N NaOH and the average value were used to normalize intracellular triptan concentrations.

Compound	Concentration (ng/mL)	Intra (n=6)			Inter (n=18)		
		Mean ± SD (ng/mL)	CV (%)	Nominal (%)	Mean ± SD (ng/mL)	CV (%)	Nominal (%)
Almotriptan	25	22 ± 4.5	20	-11.9	23.9 ± 2.7	11.3	-4.5
	100	97.5 ± 2.8	3	-7	96.1 ± 3.4	3.5	-3.9
	500	484 ± 9.7	2	-3.2	494 ± 12.9	2.6	-1.2
	5000	5229 ± 77.7	1.5	4.6	5102 ± 124	2.4	2
Eletriptan	100	106 ± 14.4	13.5	14.8	111 ± 9	8.1	11.1
	250	268 ± 10	3.7	7	258 ± 9.3	3.6	3.4
	500	508 ± 16.3	3.2	-2.1	496 ± 16	3.2	-0.8
	5000	5326 ± 163	3.1	6.5	5123 ± 188	3.7	2.5
Naratriptan	50	54.9 ± 2.4	4.4	15.8	55 ± 2.4	4.4	10
	100	103 ± 5.8	5.6	4.8	104 ± 3.9	3.8	4
	500	491 ± 20.6	4.2	-5.4	480 ± 15.8	3.3	-4
	5000	5162 ± 170	3.3	3.2	5089 ± 130	2.6	1.8
Rizatriptan	25	27.5 ± 0.9	3.4	12.2	26.8 ± 1.6	6.2	7.1
	100	98.5 ± 3.7	3.7	-1.7	99.2 ± 2.7	2.8	-0.8
	500	510 ± 10.5	2.1	-6.5	484 ± 21	4.3	-3.1
	5000	5173 ± 97.3	1.9	3.4	5024 ± 130	2.6	0.5
Sumatriptan	25	26 ± 2	7.5	13.8	26.6 ± 2	7.4	6.3
	100	103 ± 2.9	2.8	-5.2	99.2 ± 4.2	4.2	-0.8
	500	498 ± 15	3	-3.9	490 ± 14.4	2.9	-2.1
	2500	2480 ± 93	3.8	-2.2	2480 ± 70.4	2.8	-0.8
Zolmitriptan	50	47 ± 2.9	6.1	8.7	51.5 ± 4.2	8.1	3
	100	103 ± 9.4	9.2	5.7	102 ± 7	6.8	2.3
	500	498 ± 12.8	2.6	3.8	492 ± 11.8	2.4	-1.5
	5000	5113 ± 110	2.2	2.3	5052 ± 91.9	1.8	1

SD: Standard Deviation; CV: Coefficient of Variation

Table 2: Validation of HPLC-UV quantification methods of triptans.

Quantification of triptans by high-performance liquid chromatography-UV

The quantity of triptans transported in the cells was measured by high performance liquid chromatography with UV detection. The instrumentation consisted of a SpectraSystem P4000 pump, SpectraSystem AS3000 autosampler, Finnigan SpectraSystem UV6000 ultraviolet detector and SpectraSystem SN4000 system controller from Thermo Electron Corporation (San Jose, CA, USA). ChromQuest Version 4.2.34 software was used for data acquisition (Thermo Electron Corporation). The samples were separated on a Phenomenex Luna 3 μm PFP (2) column (150 × 4.6 mm, 3 μM; Phenomenex, Torrance, CA, USA). Table 1 summarizes the details for each method.

Since the cell lysate affected the absorbance of the analytes, calibration curves and quality controls samples were prepared in the

cell lysate. Linear regressions (weighted 1/concentration) were judged to produce the best fit for the concentration-detector relationship for all triptans. The coefficients of correlation (r^2) were greater than 0.997 for all compounds in all batches. The reproducibility of each method was evaluated by analyzing six replicates of lysate samples fortified at LLOQ, low, mid and high concentrations in three individual runs. Precisions were better than 11.3% and accuracies were in the 96.0-110% range. The inter- and intra-batch precision and accuracy statistical results for all compounds are shown in Table 2.

After the final wash with PBS, the samples were processed as previously described [13]. Briefly, the cells were lyzed with methanol containing the IS (100 ng/ml). The cell lysate was transferred to a 1.7 mL microtube and the samples were spun down at maximum speed for 10 min at room temperature. The supernatant was transferred to a culture borosilicate glass tube, evaporated to dryness, and reconstituted in 100 µl of reconstitution solution. The reconstitution solution consisted of a mixture of ddH_2O and methanol in the following proportions: almotriptan (70:30 v/v), eletriptan (50:50 v/v), naratriptan (70:30 v/v), rizatriptan (70:30 v/v), sumatriptan (70:30 v/v), and zolmitriptan (70:30 v/v). A volume of 20 µl per sample was injected.

Quantification of almotriptan and zolmitriptan by liquid chromatography-tandem mass spectrometry

The quantity of almotriptan and zolmitriptan transported in the cells when incubated at clinically relevant concentrations was measured by liquid chromatography-tandem mass spectrometry. The instrumentation consisted of a TSQ Quantiva Triple Quadrupole mass spectrometer interfaced with an Ultimate 3000XRS UHPLC system using pneumatic assisted heated electrospray ion source from Thermo Scientific (San Jose, CA, USA). Xcalibur 3.0.63 software was used for data acquisition and analysis (San Jose, CA, USA). The samples were separated on a Phenomenex Luna PFP (2) column (150 × 3.0 mm, 3 µM; Phenomenex, Torrance, CA, USA) coupled with a Phenomenex PFP security guard cartridge (4 × 2.0 mm; Phenomenex, Torrance, CA, USA). The mobile phase consisted of 10 mM ammonium formate, pH 3, and acetonitrile in the following proportions: almotriptan (70:30 v/v) and zolmitriptan (80:20 v/v). The flow rate was set at 0.3 ml/min and the column was heated at 40°C for almotriptan. The flow rate was set at 0.4 ml/min and the column was heated at 50°C for zolmitriptan. $^2H_6$-almotriptan and $^2H_6$-zolmitriptan were used as IS and the retention times are 4.4 and 2.8 min for almotriptan and zolmitriptan, respectively. MS detection was performed in positive ion mode, using selected reaction monitoring. The precursor-ion reactions for the analytes were set at 336.2 → 291.1 for almotriptan and 288.3 → 167.1 for zolmitriptan.

The analytical range was set at 37.5-25,000 pg/ml for almotriptan and set at 75.0-25,000 pg/ml for zolmitriptan. A linear regression (weighted 1/concentration) was judged to produce the best fit for the concentration-detector relationship for almotriptan and zolmitriptan. The r^2 was greater than 0.998 for almotriptan and 0.996 for zolmitriptan. The reproducibility of the method was evaluated by analyzing three replicates of lysate samples fortified at low, mid and high concentrations in three individual runs. Precisions were better than 13% and accuracies were in the 92-103% range. The intra and inter batch precision and accuracy statistical results are shown in Supplemental Table 1.

After the final wash with PBS, the samples were processed as follows. The cells were lyzed with 1 ml methanol containing the IS (2 ng/ml $^2H_6$-almotriptan or 0.5 ng/ml $^2H_6$-zolmitriptan). The cell lysate was transferred to a 1.7 ml microtube and the samples were spun down at maximum speed for 10 min at room temperature. The supernatant was transferred to a culture borosilicate glass tube, evaporated to dryness at 10 psi with N_2 at 40°C, and reconstituted in 200 µl of reconstitution solution. The reconstitution solution consisted of a mixture of 10 mM ammonium formate, pH 3, and methanol (70:30 v/v) for almotriptan and H_2O and methanol (95:5 v/v) for zolmitriptan. A volume of 10 µl per sample for almotriptan and 5 µl per sample for zolmitriptan was injected.

Data Analysis

The net transport of triptan through OATP1A2 was calculated by subtracting the value in the VC cells from the value in the OATP1A2 cells. Data were analyzed using GraphPad Prism 5 (GraphPad Software, La Jolla, CA, USA). Each data point is expressed as the mean ± S.D. K_m and V_{max} were calculated by fitting the data to the Michaelis-Menten equation. IC_{50} values were calculated by fitting the data to the log (inhibitor) versus response equation, and the range given represents the 95% confidence interval.

Results

Transport of triptans through OATP1A2

A cell model stably expressing OATP1A2 in HEK293 cells was used to study OATP1A2-mediated transport of the different triptans. Time-dependent uptake was assessed up to 60 min with the exception of eletriptan, up to 15 min, due to its higher lipophilicity compared to other triptans (Figure 1).

All triptans evaluated showed time-dependent saturable transport by OATP1A2. An incubation time of 2 min was chosen for further experiments with almotriptan, naratriptan, rizatriptan, sumatriptan, and zolmitriptan and 1 min was chosen for eletriptan as these time-points remain in their linear range. All triptans evaluated also showed concentration-dependent saturable transport via OATP1A2 (Figure 2 and Table 3).

The values were calculated by fitting the data to the Michaelis-Menten equation (± S.D.). CL_{int} was calculated by dividing the V_{max} by K_m. Eletriptan showed the highest affinity for OATP1A2 and sumatriptan has the lowest affinity as the K_m were calculated to be 0.8 ± 0.2 µM and 94.5 ± 9.9 µM, respectively.

OATP1A2 transport velocity was the lowest for almotriptan and the highest was observed with sumatriptan as the V_{max} were 1265 ± 54.4 pmol/mg proteins per minute and 8072 ± 300.2 pmol/mg proteins per minute, respectively. The intrinsic clearance (CL_{int}) was the lowest for sumatriptan (85.4 µl/mg protein per minute) and the highest for eletriptan (2042.5 µl/mg protein per minute).

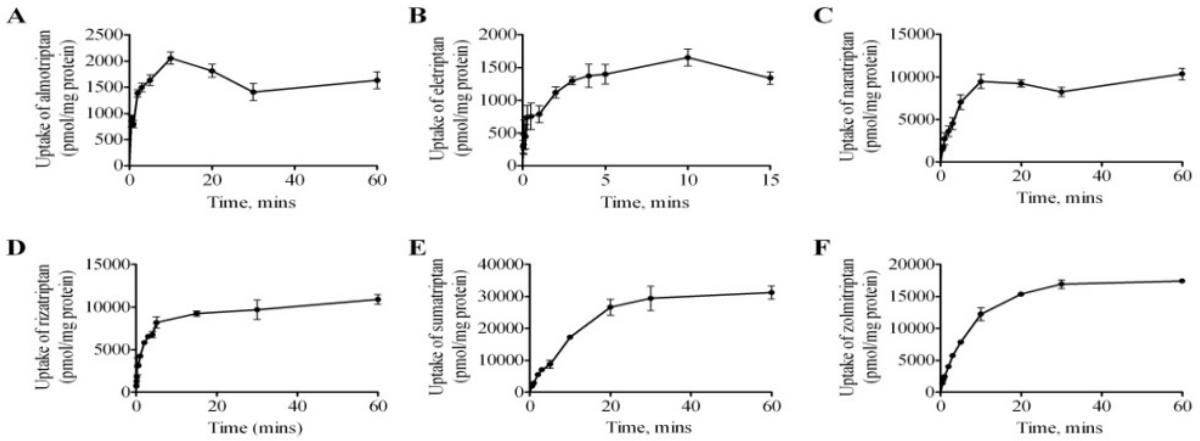

Figure 1: Time-dependent OATP1A2-mediated transport of triptans. Uptake of triptans at 37°C in HEK293-OATP1A2 and HEK293-VC cells was conducted as follows. (A) 5 μM almotriptan uptake was assessed for 0.5, 0.75, 1, 2, 3, 5, 10, 20, 30, and 60 min. (B) 1 μM eletriptan uptake was assessed for 1, 3, 5, 10, 15, and 30 s followed by 1, 2, 3, 4, 5, 10, and 15 min. (C) 20 μM naratriptan uptake was assessed for 0.5, 0.75, 1, 2, 3, 5, 10, 20, 30, and 60 min. (D) 43μM rizatriptan uptake was assessed for 1, 3, 5, 10, 15, and 30 s followed by 1, 2, 3, 4, 5, 15, 30, and 60 min. (E) 94 μM sumatriptan uptake was assessed for 0.5, 0.75, 1, 2, 3, 5, 10, 20, 30, and 60 min. (F) 21 μM zolmitriptan uptake was assessed for 0.5, 0.75, 1, 2, 3, 5, 10, 20, 30, and 60 min. The quantity of intracellular triptan was normalized to protein content. The net transport was calculated by subtracting the values measured in the VC cells from the values measured in OATP1A2 cells. Each point represents the mean ± S.D. of triplicate from a single experiment.

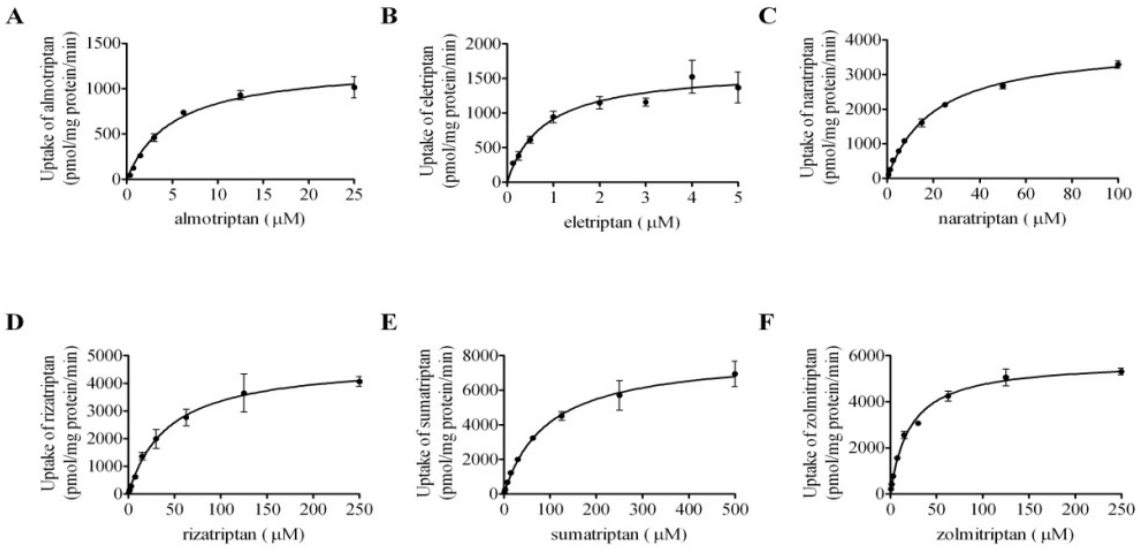

Figure 2: Km and Vmax of OATP1A2-mediated transport of triptans. Uptake of (A) almotriptan (0.375, 0.75, 1.5, 3, 6.25, 12.5, and 25 μM); (B) eletriptan (0.125, 0.25, 0.5, 1, 2, 3, 4, and 5 μM); (C) naratriptan (0.625, 1.25, 2.5, 5, 7.5, 15, 25, 50, and 100 μM); (D) rizatriptan (0.75, 1.5, 3, 7.5, 15, 30, 62.5, 125, and 250 μM); (E) sumatriptan (1.5, 3, 7.5, 15, 30, 62.5, 125, 250, and 500 μM); and (F) zolmitriptan (0.75, 1.5, 3, 7.5, 15, 30, 62.5, 125, and 250 μM) was assessed at 37°C in HEK293-OATP1A2 and HEK293-VC cells. The transport was assessed for 2 min for all triptans except for eletriptan which was assessed for 1 minute. The quantity of intracellular triptan was normalized to protein content. The net transport was calculated by subtracting the values measured in the VC cells from the values measured in OATP1A2 cells. K_m and V_{max} were calculated by fitting the data to the Michaelis-Menten equation. Each point represents the mean ± S.D. of triplicate from a single experiment.

	K_m (μM)	V_{max} (pmol/mg protein per minute)	CL_{int} (μl/mg protein per minute)
Almotriptan	5.1 ± 0.6	1265 ± 54	248
Eletriptan	0.8 ± 0.2	1634 ± 93	2042
Naratriptan	20.3 ± 1.0	3871 ± 70	191
Rizatriptan	42.9 ± 5.7	4798 ± 234	112
Sumatriptan	94.5 ± 9.9	8072 ± 300	85
Zolmitriptan	21.4 ± 1.4	5764 ± 110	269

Table 3: K_m, V_{max} and CL_{int} values for the transport of triptans through OATP1A2.

Transport of Triptans through OATP2B1

Transport of triptans through OATP2B1 was evaluated, using a HEK293 cell model stably expressing this transporter, as OATP2B1 is also found at the BBB and has overlapping substrates with OATP1A2. Two concentrations of each triptan were assessed. The concentrations chosen for each substrate correspond to its K_m and 3-times K_m value determined for OATP1A2. A slightly greater intracellular concentration of eletriptan and sumatriptan was observed in HEK293-OATP2B1 cells compared to HEK293-VC cells (Figure 3).

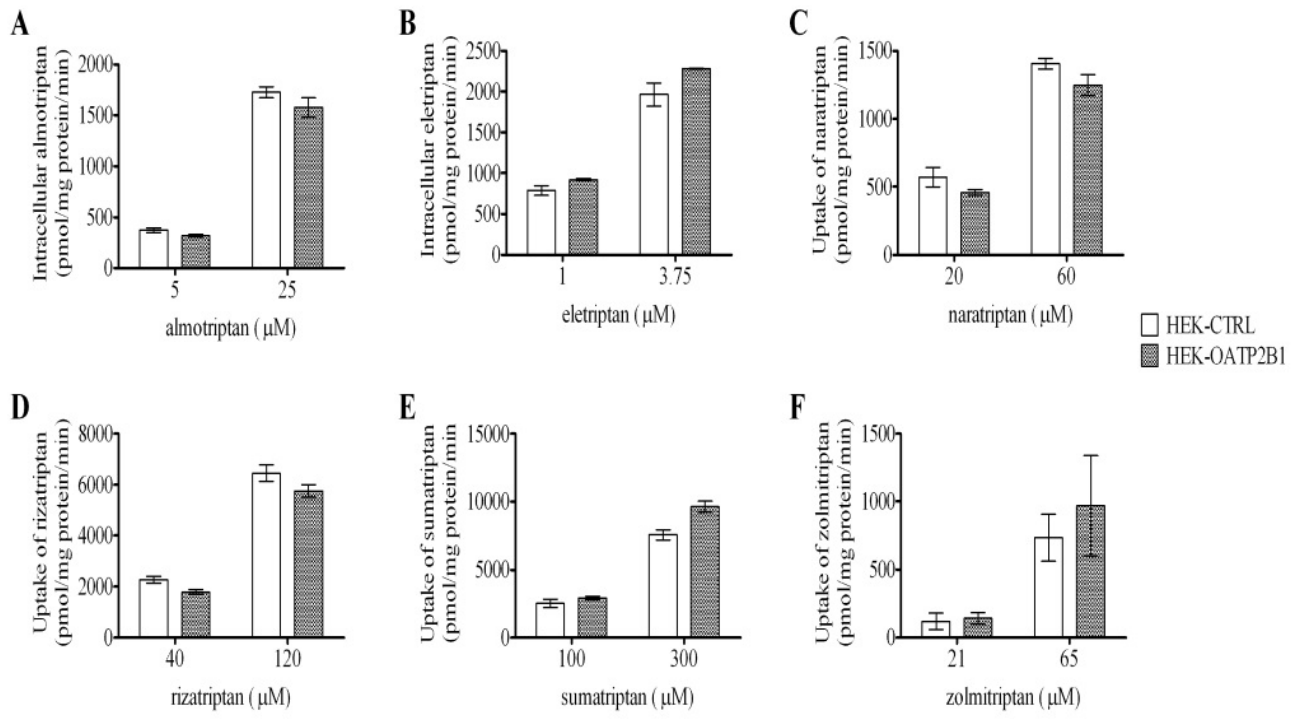

Figure 3: OATP2B1-mediated transport of triptans. Uptake of (A) almotriptan (5 and 25 μM); (B) eletriptan (1 and 3.75 μM); (C) naratriptan (20 and 60 μM); (D) rizatriptan (40 and 120 μM); (E) sumatriptan (100 and 300 μM); and (F) zolmitriptan (21 and 65 μM) was assessed at 37°C in HEK293-OATP2B1 and HEK293-VC cells. The transport was assessed for 2 min for all triptans except for eletriptan which was assessed for 1 minute. The quantity of intracellular triptan was normalized to protein content. Each point represents the mean ± S.D. of triplicate from a single experiment.

No transport by OATP2B1 was noticed when incubations were performed with almotriptan, naratriptan, rizatriptan, and zolmitriptan. The small difference, less than 21%, observed between the two cell lines with eletriptan and sumatriptan was considered non-significant and likely due to the variability of experiments.

Effect of tricyclic compounds on OATP1A2-mediated uptake of triptans

To determine whether compounds composed of a tricyclic ring and a short aliphatic amine chain inhibit OATP1A2-mediated uptake of triptans, competition studies were performed (Supplemental Figures 1-4; Table 4). Carvedilol showed the strongest inhibition on the uptake of all six triptans with an IC_{50} of 0.5, 0.7, 2.1, 2.2, 3.5, and 3.8 μM for eletriptan, almotriptan, sumatriptan, zolmitriptan, rizatriptan, and naratriptan, respectively. Carazolol was the second strongest inhibitor with an IC_{50} of 1.6, 4.6, and 5.5 μM for almotriptan, zolmitriptan, and naratriptan, respectively. amitriptyline, chlorpromazine, clomipramine, desipramine, doxepin, imipramine, nortriptyline, and trimipramine demonstrated slightly lower inhibition potencies than carvedilol and carazolol. Carbamazepine, carbazole, and phenothiazine exerted no significant effect on the transport of almotriptan, naratriptan, and zolmitriptan.

	Almotriptan	Naratriptan	Zolmitriptan	Eletriptan	Rizatriptan	Sumatriptan
Inhibitors	μM					
Amitriptyline	4.6 (2.4–8.9)	13.2 (7.2–24.2)	6.4 (4.0–10.1)	N/A	12.6 (6.8–23.4)	9.5 (5.2–17.4)
Carazolol	1.6 (0.9–2.8)	5.5 (3.3–9.2)	4.6 (3.1–6.8)	N/A	N/A	N/A
Carvedilol	0.7 (0.3–1.4)	3.8 (3.0–4.8)	2.2 (1.6–2.9)	0.5 (0.2–1.6)	3.5 (2.2–5.6)	2.1 (1.2–3.6)
Chlorpromazine	8.7 (4.9–15.6)	20.3 (12.1–34.1)	16.9 (10.2–27.8)	N/A	N/A	N/A
Clomipramine	6.1 (3.7–10.2)	19.6 (11.4–33.9)	13.5 (8.8–20.9)	N/A	N/A	N/A
Desipramine	16.2 (7.5–35.0)	19.8 (11.1–35.3)	18.4 (12.1–28.1)	N/A	N/A	N/A
Doxepin	2.5 (1.7–3.9)	12.9 (8.4–19.8)	6.8 (2.9–16.1)	N/A	4.8 (2.2–10.8)	5.9 (3.4–10.2)
Imipramine	4.3 (2.8–6.7)	7.4 (3.2–17.1)	10.3 (4.7–22.4)	11.1 (2.7–46.1)	N/A	N/A
Nortriptyline	4.5 (2.6–7.8)	19.1 (6.8–53.7)	13.0 (9.4–17.9)	81.0 (9.9–662.2)	N/A	N/A
Trimipramine	7.6 (3.5–16.4)	20.0 (12.2–32.6)	13.6 (9.4–19.7)	N/A	N/A	N/A
Carbamazepine	No effect	No effect	No effect	N/A	N/A	N/A
Carbazole	No effect	No effect	No effect	N/A	N/A	N/A
Phenothiazine	No effect	No effect	No effect	N/A	N/A	N/A
N/A: Not available (The inhibition assay was not evaluated)						

Table 4: IC_{50} values from the inhibition of triptans uptake through OATP1A2 by different tricyclic compounds. The values in parentheses represent the 95% confidence interval (Supplemental Figures 1-4).

Studies in the range of total plasma concentrations

As the IC_{50} studies were carried out with the concentration of substrates at saturation, it does not reflect the interaction at the BBB in clinical settings. Thus, a study using range of total plasma concentrations of triptans and inhibitors were carried out with almotriptan and zolmitrptan. These two triptans were selected based on their greater hydrophilic profile in the cell model used. In Canada, almotriptan is typically given in a 12.5 mg dose tablet and zolmitriptan is typically given in a 2.5 mg dose tablet, 2.5-5 mg dose nasal spray or 2.5 mg dose orally disintegrating tablet [14]. Pharmacokinetic studies have shown that a single dose of almotriptan results in a C_{max} of 50 ng/mL and a single dose of zolmitriptan in any of the dosage forms corresponds to a C_{max} of 3-7 ng/mL [15-19].

Time-dependent uptake of almotriptan and zolmitriptan was re-assessed as lower concentrations might affect the kinetics. almotriptan (50 ng/mL) and zolmitriptan (3 ng/mL) showed a time-dependent saturable transport with a similar profile as when incubated at K_m (Figures 4 and 5).

An incubation time of 1 and 2 min was chosen for the competition experiments for almotriptan and zolmitriptan, respectively. The concentrations of inhibitors correspond to the plasma concentrations measured at half C_{max}, C_{max}, and 2-times C_{max} for a given dose. The reported peak plasma concentrations (C_{max}) after an oral dose are: 33.5 ng/ml for amitriptyline 50 mg; 35 ng/ml for carvedilol 12.5 mg; 50 ng/ml for chlorpromazine 100 mg; 63 ng/ml for clomipramine 25 mg; 18 ng/ml for desipramine 50 mg; 25 ng/ml for doxepin 75 mg; 63 ng/ml for imipramine 100 mg; 50-150 ng/ml for nortriptyline 25-50 mg; and 22 ng/ml for trimipramine 75 mg [20-28]. Among the inhibitors evaluated, carvedilol and nortriptyline lowered the uptake of both almotriptan and zolmitriptan whereas clomipramine diminished the uptake of almotriptan only. The other tricyclic compounds had no significant effects on OATP1A2-mediated uptake of almotriptan and zolmitriptan at clinically relevant concentrations.

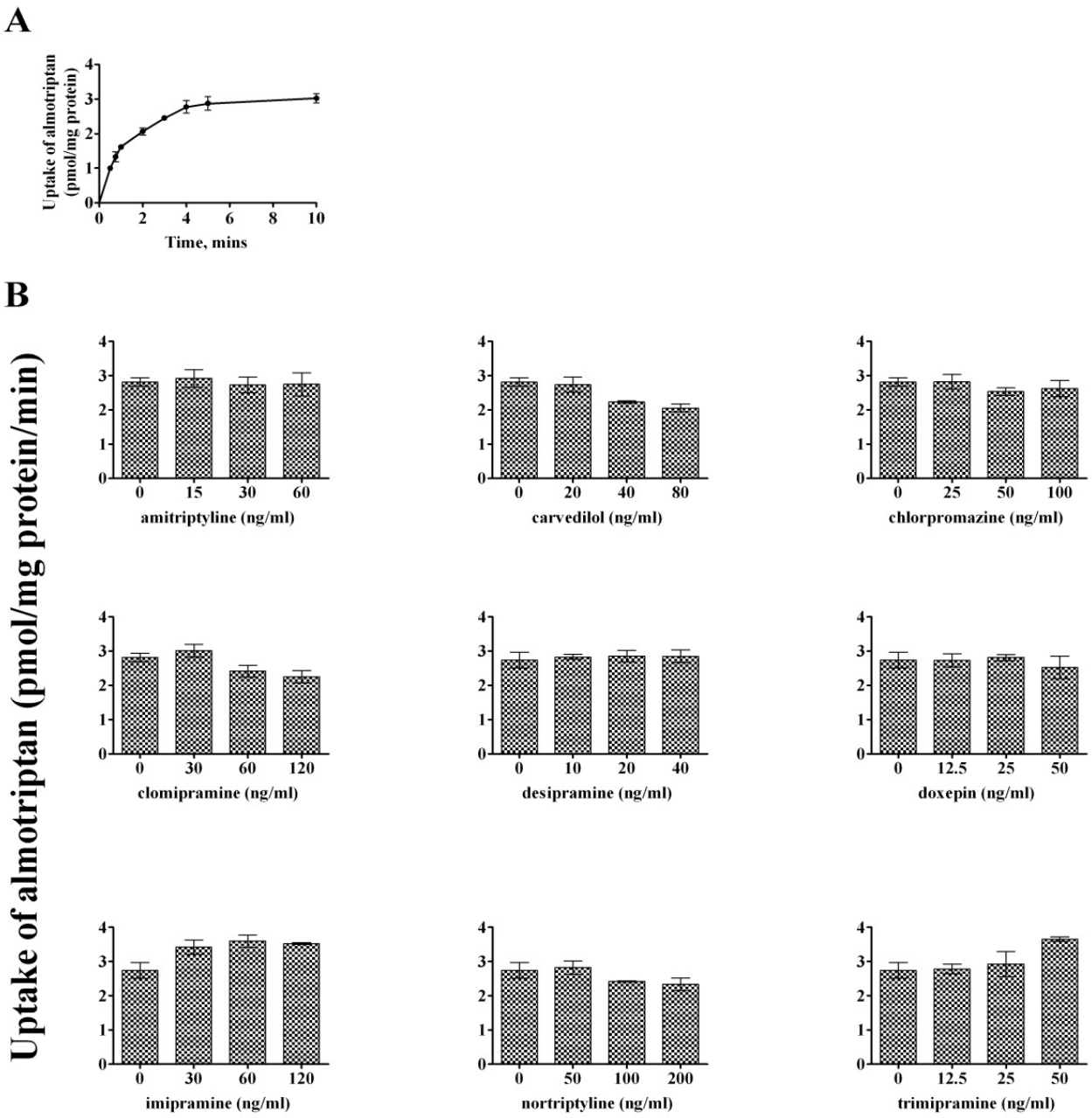

Figure 4: Inhibition of OATP1A2-mediated transport of almotriptan by various tricyclic compounds at range of total plasma concentrations of the drugs. (A) The uptake of almotriptan (50 ng/ml) was assessed for 0.5, 0.75, 1, 2, 3, 4, 5, and 10 min at 37°C in HEK293-OATP1A2 and HEK293-VC cells. (B) HEK293-OATP1A2 and HEK293-VC cells were co-incubated with almotriptan (50 ng/ml) and different tricyclic compounds (10-200 ng/ml) for 1 min at 37°C. The quantity of intracellular almotriptan was normalized to protein content. To obtain the net transport, values measured in the VC cells were subtracted from the values measured in OATP1A2-expressed cells. Each point represents the mean ± S.D. of triplicate from a single experiment.

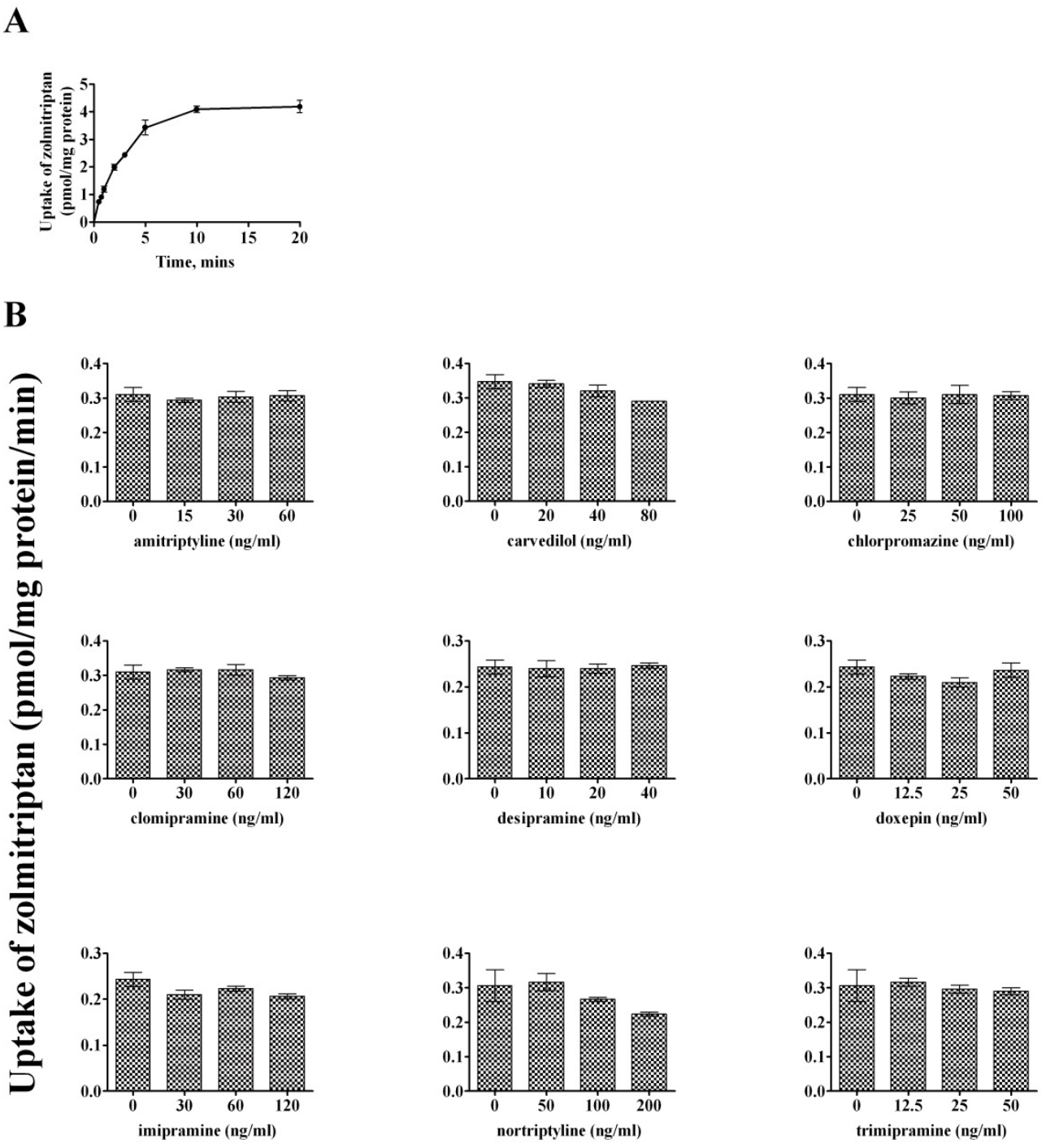

Figure 5: Inhibition of OATP1A2-mediated transport of zolmitriptan by various tricyclic compounds at range of total plasma concentrations of the drugs. (A) The uptake of zolmitriptan (3 ng/ml) was assessed for 0.5, 0.75, 1, 2, 3, 5, 10, and 20 min at 37°C in HEK293-OATP1A2 and HEK293-VC cells. (B) HEK293-OATP1A2 and HEK293-VC cells were co-incubated with zolmitriptan (3 ng/ml) and different tricyclic compounds (10-200 ng/ml) for 2 min at 37°C. The quantity of intracellular zolmitriptan was normalized to protein content. To obtain the net transport, values measured in the VC cells were subtracted from the values measured in OATP1A2-expressed cells. Each point represents the mean ± S.D. of triplicate from a single experiment.

Discussion

Using stable cell lines overexpressing selected transporters, we confirmed that triptans are substrates for OATP1A2 but not OATP2B1. Inhibition studies demonstrated that compounds composed of a tricyclic ring and a short aliphatic amine chain inhibited OATP1A2-mediated uptake of triptans. The IC_{50} values of the inhibitors determined in this study followed the same pattern as those previously published when using rosuvastatin as the probe substrate for OATP1A2 [13]. Carvedilol and carazolol were the strongest inhibitors followed by amitriptyline, chlorpromazine, clomipramine, desipramine, doxepin, imipramine, nortriptyline, and trimipramine. Inhibition studies conducted in the range of total plasma concentrations showed that carvedilol, clomipramine and nortriptyline were able to diminish the transport of triptans through OATP1A2.

The K_m values of almotriptan, eletriptan and zolmitriptan for OATP1A2 determined in this study (5.1, 0.8, and 21.4 μM, respectively) are in line with those previously published (4.8, 1.3, and 15.1 μM, respectively) [12]. However, the K_m values of rizatriptan and sumatriptan are higher in this study (42.9 and 94.5 vs. 6.0 and 27.0 μM). The drug's solubility in the solvent used to dissolve or the incubation buffer may account for this discrepancy. In fact, when a drug is incompletely dissolved, the shape of the K_m V_{max} curve is changed when compared to the situation where the drug is completely dissolved at all concentrations. The V_{max} values for the substrates are higher in this study. This variability may be explained by the differences in the *in vitro* model used: the quantity of OATP1A2 protein expressed at the cell surface, the quantity of functional proteins expressed, or the quantity of transporters exposed to the media and available for drug uptake. Although the CL_{int} values (V_{max}/K_m) are different in the two studies, they both follow the same order of magnitude: eletriptan>zolmitriptan>almotriptan>rizatriptan> sumatriptan. Naratriptan could not be compared as the previous publication did not assess it.

There are evidences supporting a mechanism of action in the CNS for triptans in addition to their peripheral effects: 1) 5-HT_{1B} and 5-HT_{1D} receptors proteins are found on trigeminal sensory neurons; and 2) Activation of the trigeminal nucleus neurons by electrical stimulation is inhibited after administration of a triptan in animal models [2,3,29-31]. CNS adverse events, such as dizziness, vertigo, and ataxia are indirect indications that triptans have the potential to access the brain [32]. Using positron emission tomography (PET), two studies demonstrated that zolmitriptan can penetrate the brain at therapeutic doses and can bind to their receptors located in the CNS [33,34]. As triptans are hydrophilic, thus cannot cross the BBB by passive diffusion, OATP1A2 may play a role in facilitating the transport of triptans to their site of action. Our data suggest that the co-administration of carvedilol, clomipramine, or nortriptyline with a triptan may limit the entrance of triptans to the CNS by inhibiting OATP1A2. The drug concentration might fall below its therapeutic window in the brain. Consequently, the antimigraine activity may be abolished.

Of interest, one third of migraineurs receiving triptan therapy do not achieve headache relief and the most common reason for the discontinuation of these medications is the lack efficacy [32,35,36]. With the purpose of understanding the causes behind this lack of efficacy, a few studies have looked at polymorphisms found in genes involved in the pharmacokinetic and pharmacodynamic response to triptans. Associations have been reported for the genes encoding the serotonin transporter, monoamine oxidase A, and CYP1A2 [37]. Polymorphisms in the gene encoding for OATP1A2 and their ability to transport triptans should also be investigated. However, pharmacogenomics alone may not explain the lack of efficacy of triptans in all non-responders. Drug-drug interactions may explain inter-subject variability in antimigraine efficacy for cases where gene polymorphisms are not involved. Interestingly, migraine is often diagnosed in patients with mood disorders, such as depression, anxiety, panic disorder, and bipolar disorder [38,39]. As a result, treatments for both conditions are commonly prescribed. Tricyclic antidepressants are not only prescribed for depression but also for other off-label uses such as obsessive-compulsive disorder, panic disorder, chronic pain, insomnia, premenstrual symptoms and bulimia. In addition, β-blockers and antidepressants, especially amitriptyline, are occasionally prescribed for the prevention of migraine attacks [40]. These observations indicate that the co-prescription of a triptan with a tricyclic antidepressant is not unusual.

Taken together, we demonstrated that compounds composed of a tricyclic ring and a short aliphatic amine chain inhibited the OATP1A2-mediated uptake of triptans. Our data suggest that carvedilol, clomipramine, and nortriptyline may limit the penetration of triptans to the brain by modulating OATP1A2 transport. Although an *in vitro* cell model permits to study the transport of a drug through a specific transporter, this experimental model is also associated with limitations when extrapolating *in vitro* findings to *in vivo* settings. Thus, emphasizing the need to confirm these results in humans. Indeed, the impact of concomitant administration of triptans with a potent OATP1A2 inhibitor on their antimigraine efficiency needs to be investigated further in clinical studies.

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