Interactions of antiretroviral drugs with the SLC22A1 (OCT1) drug transporter

Session: P-H3
Board #: 519

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INTRODUCTION

• SLC22A1 (OCT1) is expressed on the basolateral membrane of hepatic liver cells and is involved in the excretion of numerous cationic drugs into the bile.[1]

• The inhibition of SLC22A1 by several antiretrovirals, such as the protease inhibitor darunavir, has not previously been determined.

• In order to better understand and predict drug-SLC22A1 interactions, a range of antiretrovirals were screened for SLC22A1-associated inhibition and transport.

METHODS

• Stable SLC22A1-expressing KCL22 cells were produced previously by nucleofection and neomycin selection (RPMI, 10% [vol/vol] FCS, 37ºC, 5% CO_2).[2]

• Control KCL22 cells were transfected with the empty vector pcDNA3.1.

• Accumulation of ^{14}C radiolabelled tetraethylammonium (TEA; 5.4 µM, 0.3 µCi/mL) was determined in SLC22A1-expressing and mock-transfected KCL22 cells with and without 50 µM of control SLC22A1 inhibitors (prazosin or cepharanthine), or 50 µM of each antiretroviral drug, following incubation (1 mL, 2.5 x 10^6 cells/mL, 37ºC, 5% CO_2, RPMI, 10% [vol/vol] FCS, 30 min).

• The antiretroviral drugs examined as potential SLC22A1 inhibitors were atazanavir, lopinavir, amprenavir, indinavir, darunavir, ritonavir, nelfinavir, lamivudine, tenofovir, zalcitabine, abacavir, zidovudine, stavudine, etravirine, nevirapine, efavirenz, rilpivirine and raltegravir.

• Following this experiment, the procedure was repeated with modifications to calculate SLC22A1 IC_{50} values for selected antiretrovirals. Accumulation of [^{14}C]TEA (5.4 µM, 0.3 µCi/mL) was determined when cells were co-incubated with a log range of concentrations (0, 1, 2.5, 5, 10, 25, 50, 100 µM) of either control SLC22A1 inhibitor prazosin, protease inhibitor darunavir, or non-nucleoside reverse transcriptase inhibitor efavirenz.

• Drug concentrations were analysed using radioactive scintillation and statistics were performed using SPSS. Normality was determined using the Shapiro-Wilk test. An independent T test was used for normally distributed data and a Mann-Whitney test was used for non-normally distributed data. P values less than 0.05 were deemed significant.

RESULTS

Drug class	Inhibitor	TEA in cells (µM ± SD)	P value
No inhibitors	None	10.5 ± 0.6	n/a
	Mock transfected	0.3 ± 0.004	<0.001
Control SLC22A1 inhibitors	Prazosin	2.1 ± 0.4	<0.001
	Cepharanthine	2.4 ± 0.2	<0.001
Protease inhibitors	Atazanazir	10.2 ± 0.8	0.649
	Lopinavir	7.2 ± 1.8	0.018
	Amprenavir	6.8 ± 1.6	0.007
	Indinavir	5.2 ± 0.6	<0.001
	Darunavir	4.7 ± 0.4	<0.001
	Ritonavir	4.0 ± 0.3	<0.001
	Nelfinavir	3.1 ± 2.5	0.033
Nucleoside reverse transcriptase inhibitors	Lamivudine	11.4 ± 0.6	0.083
	Tenofovir	9.7 ± 0.6	0.158
	Zidovudine	8.5 ± 3.1	0.390
	Zalcitabine	9.1 ± 0.7	0.042
	Abacavir	8.8 ± 0.8	0.027
	Stavudine	7.6 ± 0.6	0.001
Non-nucleoside reverse transcriptase inhibitors	Etravirine	8.2 ± 1.4	0.032
	Neviripine	8.0 ± 1.5	0.026
	Rilpivirine	6.3 ± 0.2	<0.001
	Efavirenz	5.4 ± 0.7	<0.001
Integrase inhibitors	Raltegravir	9.6 ± 1.4	0.340

Table 1) The effects of protease inhibitors, nucleoside reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitors and the integrase inhibitor raltegravir on the accumulation of tetraethyl ammonium (TEA) in KCL22 cells (µM of TEA in cells, 30 min incubation, n=3 experimental reps) ± SD. Apart from the no inhibitor experiment, P values represent the statistical difference of the condition with the result obtained using SLC22A1-expressing KCL22 cells with and without inhibitor. For the no inhibitor experiment P value represents differences between SLC22A1-expressing and mock-transfected cells.

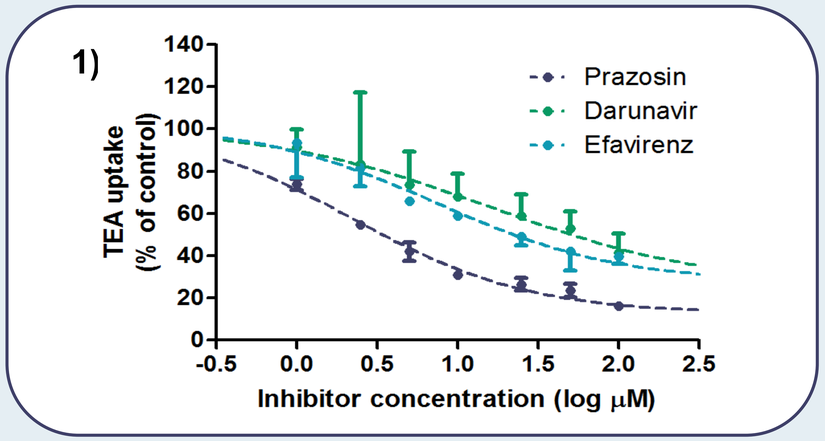

Figure 1) Inhibitory concentrations (IC_{50}) for control SLC22A1 inhibitor prazosin, protease inhibitor darunavir and non-nucleoside inhibitor efavirenz (given as percentage of TEA in cells compared to TEA in cells with no inhibitor, 30 min incubation, n=3 experimental reps) for SLC22A1-mediated transport of TEA. Data are given as mean ± SD.

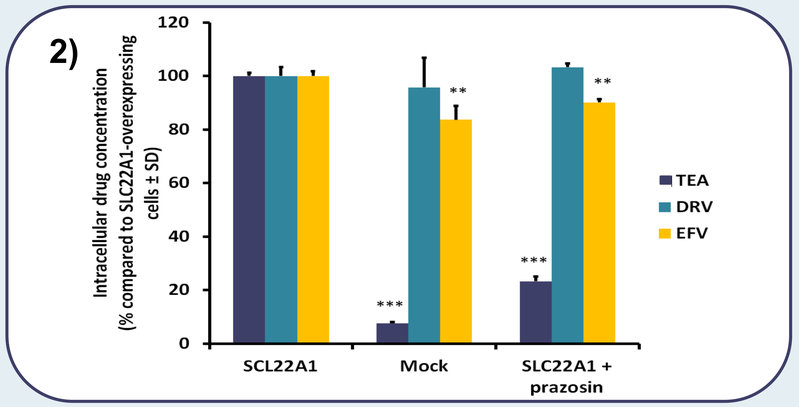

Figure 2) The accumulation of efavirenz and darunavir in mock-transfected KCL22 cells, SLC22A1-overexpressing KCL22 cells and SLC22A1-overexpressing cells co-incubated with prazosin. The amount of drug in mock transfected cells and SLC22A1-overexpressing KCL22 cells subjected to prazosin were compared to SLC22A1-overexpressing KCL22 cells (% compared to SLC22A1 overexpressing cells ± SD, n = 3 experimental replicates). ** = $p < 0.01$, *** = $p < 0.001$.

• TEA accumulation was higher in SLC22A1-expressing cells compared to mock-transfected cells (10.5 ± 0.6 µM versus 0.3 ± 0.004 µM, $p<0.001$) and was significantly reduced in SLC22A1-expressing cells when co-incubated with all antiretrovirals tested except atazanavir, lamivudine, tenofovir, zidovudine and raltegravir (Table 1).

• SLC22A1 IC_{50} values in the presence of 10% FBS for efavirenz, darunavir and prazosin were 21.8, 46.2 and 2.8 µM, respectively (Figure 1).

• Efavirenz accumulation was higher in SLC22A1-expressing cells compared to mock-transfected cells (16.3% higher, $p<0.01$) which was reversed using prazosin, whereas no difference was observed for darunavir ($p=0.45$) (Figure 2).

CONCLUSIONS

• Numerous antiretrovirals showed inhibition of SLC22A1 in vitro. Particularly noticeable was the predominance of SLC22A1 inhibitors in the protease inhibitor and non-nucleoside reverse transcriptase inhibitor classes.

• Maximum patient plasma concentrations of efavirenz (12.9 µM) and darunavir/r (11.9 µM) are lower than the SLC22A1 IC_{50}s determined.

• Efavirenz showed only moderately higher but statistically significant accumulation in SLC22A1-expressing cells.

• These data inform the mechanistic basis for disposition, drug-drug interactions and pharmacogenetic candidate gene selection for antiretroviral drugs.

REFERENCES

[1] Jung *et al. Drug Metab Dispos* 2008; 36(8):1616-23
[2] Giannoudis *et al. Blood* 2008; 112(8):3348-54