EFFECT OF INFLAMMATION ON DRUG TRANSPORTER CONCENTRATIONS IN HUMAN AIRWAY EPITHELIA MEASURED BY QUANTITATIVE TARGETED ABSOLUTE PROTEOMICS

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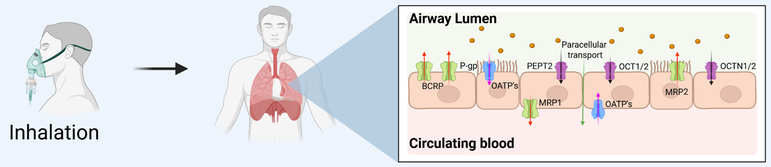
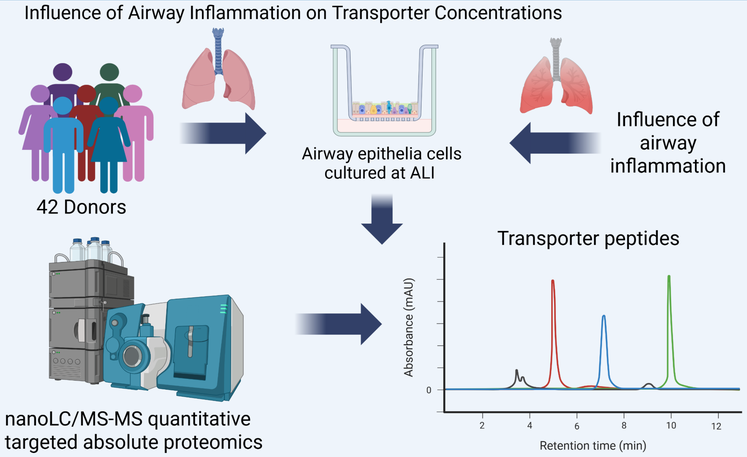
VISUAL ABSTRACT

Inhaled Drugs and Lung Transporters



Study Aim

Influence of Airway Inflammation on Transporter Concentrations



METHODS

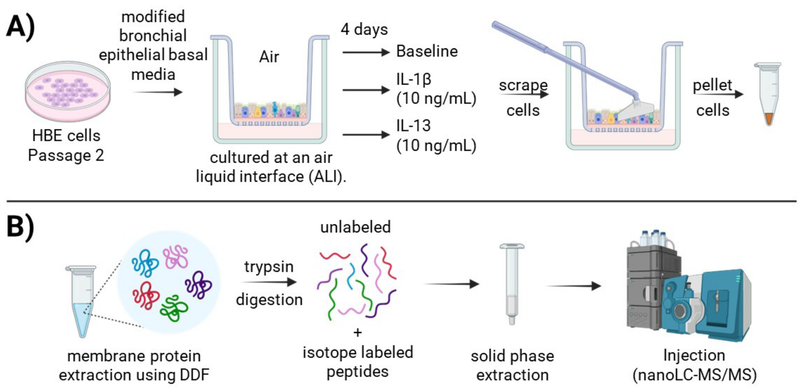


Figure 1. A) Human Bronchial Epithelia (HBE) Culture[1,2] and B) Quantitative Targeted Absolute Proteomic (QTAP) Analysis[3,4] Workflow. Separate HBE cultures from each donor cultured at an ALI were exposed to 10 ng/mL IL-1β (surrogate for TH1/neutrophilic inflammation) or 10 ng/mL IL-13 (surrogate for TH2/eosinophilic inflammation) for four days, and duplicate cultures from each inflammatory condition were scraped, combined, and stored as above. ALI, air liquid interface; IL, interleukin; DDF, differential detergent fractionation. Created with BioRender.com (also visual abstract).

Table 1: Donor Demographics.

	Large Airway
N =	42
Sex, male (%)	20 (48%)
Age in years, (mean ± SD)	34.8 ± 18.6
Age range in years (min – max)	12-73
Race/ethnicity	
White (%)	21 (50%)
Black (%)	9 (21%)
Hispanic (%)	11 (26%)
Pacific Islander (%)	1 (2%)

RESULTS

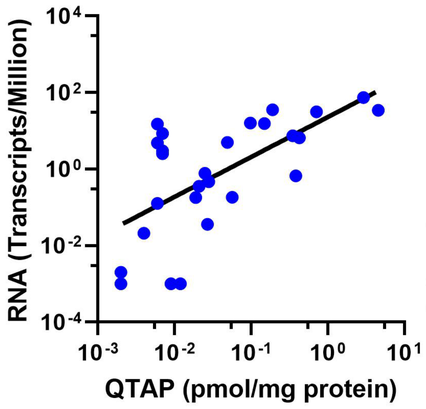


Figure 2. Relationship between mean concentrations (pmol/mg membrane protein) of transport proteins in HBE as measured by QTAP to RNA expression (transcripts/million) of the corresponding genes measured by RNA sequencing in a prior study[5]. Linear regression R^2 = 0.41, p<0.003. N = 31 proteins.

Table 2: Transporter Protein Concentrations and mRNA Expression in Human Bronchial Epithelia (HBE) cell membranes at Baseline.

Gene	Protein	# of Donors in which detected	QTAP* (pmol/mg Protein)	RNA (Transcripts/Million)
ABCB1	P-gp	1	N.D.	0.00±0.01
ABCG2	BCRP	8	0.044±0.082 (<LLOQ)	0.18±0.15
	BCRP*2	1	N.D.	
ABCC1	MRP1	42	3.955±1.638	34.3±7.5
ABCC2	MRP2	1	N.D.	0.78±0.30
ABCC3	MRP3	10	0.056±0.103 (<LLOQ)	4.99±1.11
ABCC4	MRP4	42	0.385±0.160	7.40±2.81
ABCC5	MRP5	20	0.239±0.378	35.6±4.0
ABCC6	MRP6	13	0.174±0.307	15.5±5.4
ABCC12	MRP9	1	N.D.	0.00±0.00
SLC15A2	PEPT2	42	3.124±0.948	74.3±18.6
SLC22A7	OAT2	1	N.D.	0.00±0.00
SLC22A8	OAT3	0	N.D.	0.00±0.00
SLC22A1	OCT1	0	N.D.	0.13±0.09
SLC22A2	OCT2	0	N.D.	0.00±0.00
SLC22A3	OCT3	6	0.049±0.109 (<LLOQ)	0.47±0.59
SLC22A4	OCTN1	41	0.707±0.338	31.2±13.2
SLC22A5	OCTN2	2	N.D.	15.0±1.5
SLC28A1	CNT1	4	0.038±0.133 (<LLOQ)	0.18±0.19
SLC28A3	CNT3	1	0.084±0.537 (<LLOQ)	0.67±0.16
SLC29A1	ENT1	11	0.646±1.357	6.53±1.27
SLC29A2	ENT2	2	0.027±0.124	8.45±1.12
SLC29A4	ENT4	0	N.D.	0.31±0.15
SLC47A1	MATE1	4	N.D.	0.91±0.36
SLC47A2	MATE2	2	N.D.	2.98±0.77
	MATE2-K	3	0.030±0.121 (<LLOQ)	
SLCO1A2	OATP1A2	1	N.D.	0.04±0.04
SLCO2A1	OATP2A1	1	N.D.	2.53±3.21
SLCO1B1	OATP1B1	0	N.D.	0.02±0.03
SLCO1B3	OATP1B3	1	N.D.	4.79±2.52
SLCO2B1	OATP2B1	1	N.D.	0.00±0.00
SLCO4C1	OATP4C1	14	0.093±0.139 (<LLOQ)	16.1±3.2

*Transporter protein concentrations are those measured in this study (LLOQ, lower limit of quantification of 0.1 pmol/mg protein and LOD, limit of detection of 0.02 pmol/mg protein). N.D., not detected. Transporter mRNA values were obtained from Gene Expression Omnibus that included results from Mikami et al. (2023)[5].

Table 3: Multivariate analysis (N = 42 donors).

	MRP1		MRP4		MRP5		OCTN1	
	β ± SE	p-value	β ± SE	p-value	β ± SE	p-value	β ± SE	p-value
Black	0.15 ± 0.66	0.820	0.11 ± 0.06	0.080	0.10 ± 0.14	0.505	-0.06 ± 0.13	0.647
Hispanic	-0.04 ± 0.61	0.950	0.03 ± 0.06	0.611	-0.02 ± 0.13	0.872	0.10 ± 0.12	0.389
Sex	0.24 ± 0.51	0.650	**0.11 ± 0.05**	**0.027**	0.07 ± 0.11	0.504	0.11 ± 0.10	0.262
Age	0.025 ± 0.014	0.081	0.001 ± 0.001	0.393	**0.008 ± 0.003**	**0.010**	**-0.007 ± 0.003**	**0.016**

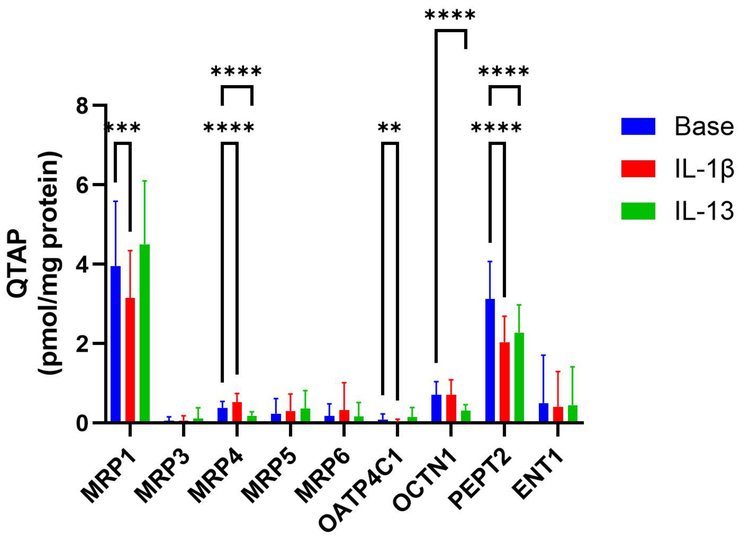


Figure 3. Protein concentrations (pmol/mg membrane protein) of transporters expressed in HBE under baseline conditions (baseline, blue bars) and after 4-day exposure to 10 ng/mL IL-1β (red bars) or IL-13 (green bars). Concentrations of the highly expressed MRP1 and PEPT2 transporters were altered by IL-1β and IL-13 exposure, as were those of several other transporters. Data are shown as mean and standard deviation (n=42, *=p<0.05, **=p<0.01, ***=p<0.001, ****=0.0001).

SUMMARY & CONCLUSIONS

- Transporters have been quantified in HBE cells cultured from a broad demographic of lung donors. Protein concentrations correlate significantly with mRNA expression.
- MRP1 and PEPT2 are the most highly expressed transporters in HBE. Both were reduced significantly after exposure in vitro to IL-1β. MRP1 increased after exposure to IL-13 and PEPT2 decreased, with just the PEPT2 decrease being significant.
- Multivariate analysis showed sex and age to impact the concentrations of select transporters.
- Our study demonstrates that inflammation impacts transporter concentrations in a way that could influence the pharmacokinetics of inhalation xenobiotics.

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REFERENCES

1) Fulcher & Randell (2013), *Methods Mol Biol* 945:109-121 (ALI Culture).
2) Gentzsch et al. (2021), *Front Pharmacol* 12:628722 (Inflammation Studies).
3) Qasem et al. (2021), *J Pharm Sci* 110(1):87-96 (Detergent Fractionation).
4) Fashe et al. (2022), *Front Pharmacol* 13:1004010 (Transporters QTAP).
5) Mikami et al. (2023), *Sci Transl Med* 15(699):eabo7728 (Transporter RNA).