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in vitro Solutions for Respiratory Diseases and Chemical Testing

Development of a human airway tissue-based assay for respiratory absorption giving input parameters for PBTK modelling

Samuel Constant[(1)], Wiebke Hoffmann[(2)], Julieta Gradinaru[(3)], Lucian R. Farcal[(2)], Ludovic Wiszniewski[(1)], Song Huang[(1)], Pierre-Alain Carrupt[(3)], Sandra Coecke[(2)]

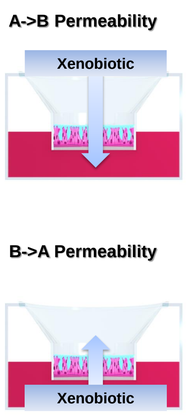
(1) Epithelix Sàrl, 14 chemin des aulx, CH-1228 Plan-les-Ouates, Switzerland, email: epithelix@epithelix.com

(2) European Commission - DG Joint Research Centre, Institute for Health and Consumer Protection, Systems Toxicology Unit, The European Union Reference Laboratory for Alternatives to Animal Testing (EURL ECVAM), Ispra, Italy

(3) School of Pharmaceutical Sciences, Université de Genève, Genève, Switzerland

The respiratory system is a prime entry portal of xenobiotics into the body. Knowledge of toxicokinetics is needed to estimate the possible range of target doses at the cell or tissue level that can be expected from realistic human exposure scenarios to inhaled compounds. The upper respiratory tracts, especially the nasal cavity, are main deposition sites of particles and volatile compounds. Nasal cavity is also considered as an ideal route for drug delivery. Indeed an increasing number of drugs are administrated via nasal cavity. Therefore, it is important to understand how chemicals interact with and pass through the upper airway epithelia. Our study evaluated the permeability property of an *in vitro* cell model of the human airway epithelium (MucilAir™). A standard operating procedure was developed and its transferability and reproducibility was evaluated.

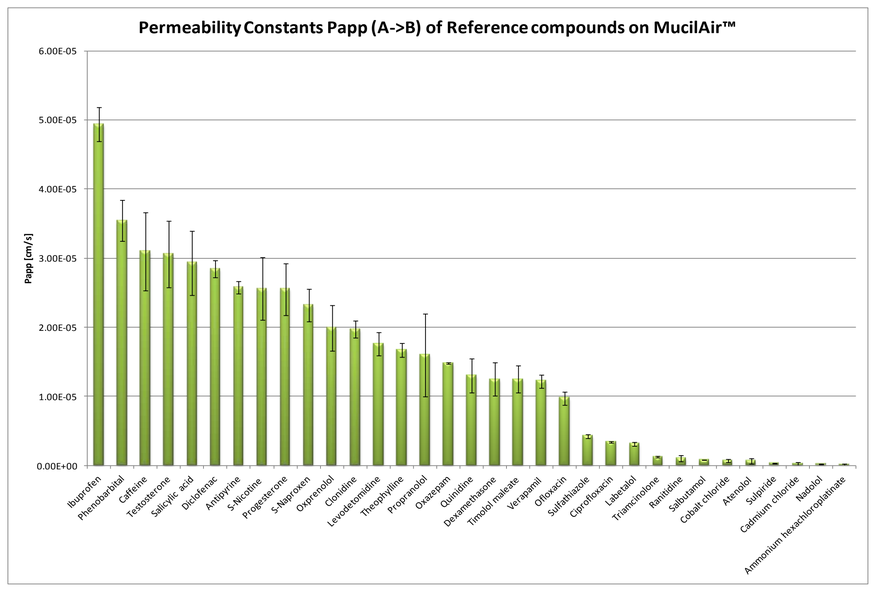
Trans epithelial transport of xenobiotics



Molecules	Papp (cm/s) A→B	Papp (cm/s) B→A	Asymmetry Ratio
Salicilic Acid	7.7×10^{-5}	1.7×10^{-5}	0.2
Nicotine	2.1×10^{-5}	3.3×10^{-5}	1.6
Propranolol.HCl	1.2×10^{-5}	1.6×10^{-5}	1.3
Ibuprofen	1.1×10^{-5}	1.9×10^{-5}	1.7
Tripolidine.HCl	9.7×10^{-6}	1.2×10^{-5}	1.2
Tetracaïne.HCl	8.0×10^{-6}	1.1×10^{-5}	1.3
Dopamine.HCl	3.0×10^{-6}	2.5×10^{-6}	0.8
Atenolol	2.2×10^{-6}	6.7×10^{-6}	3.0

The absorption of test items was assessed after apical or basolateral exposures, and the permeability rate (Papp) of the chemicals across airway epithelium was measured.

Papp (A->B) of 30 reference compounds



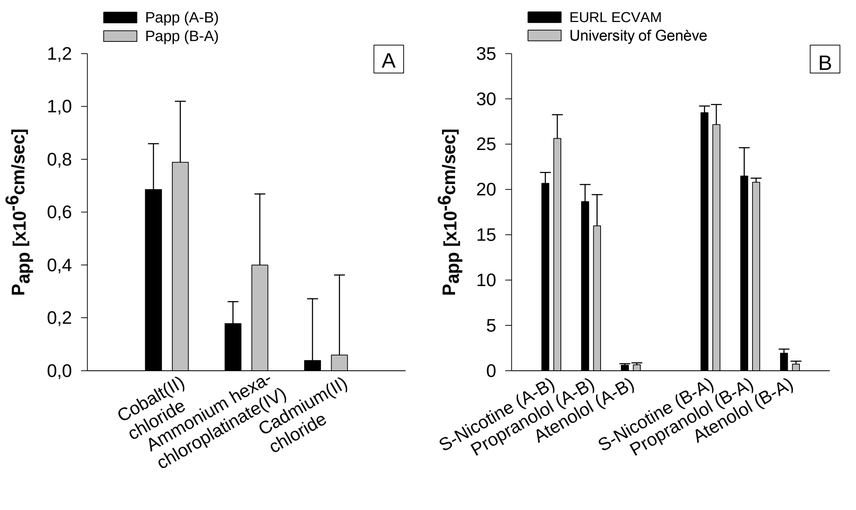
A panel of 30 compounds were further tested to evaluate the ability of the assay to rank relative permeability (Papp A->B from 2.06E-07 to 4.94E-05 and Papp B->A from 4.50E-07 to 3.44E-05 cm/s).

Intranasal/intrabronchial permeability

Batch	Age of the MucilAir™ (months)	TEER Before exp (Ω.cm²)	TEER After Exp (Ω.cm²)	Papp (cm/s) A→B
Nasal-MucilAir™				
Pol 41	13	554 ± 103	442 ± 33	$3.97\ 10^{-5} \pm 4.32\ 10^{-7}$
Pol 43	12	469 ± 9	355 ± 54	$3.80\ 10^{-5} \pm 2.06\ 10^{-6}$
Pol 49	9	615 ± 35	340 ± 14	$3.02\ 10^{-5} \pm 1.34\ 10^{-6}$
Pol 51	6	376 ± 65	256 ± 30	$2.88\ 10^{-5} \pm 2.41\ 10^{-6}$
Pol 52	6	396 ± 14	332 ± 40	$1.66\ 10^{-5} \pm 6.93\ 10^{-6}$
Pol 53	6	495 ± 23	439 ± 75	$1.22\ 10^{-5} \pm 5.37\ 10^{-7}$
Pol 55	2	589 ± 2	685 ± 35	$2.23\ 10^{-5} \pm 1.18\ 10^{-5}$
Pol 57	3	480 ± 35	294 ± 37	$2.11\ 10^{-5} \pm 9.49\ 10^{-6}$
Pol 58	2	413 ± 28	406 ± 14	$2.20\ 10^{-5} \pm 1.12\ 10^{-5}$
Pol 59	2	375 ± 2	338 ± 2	$1.86\ 10^{-5} \pm 9.52\ 10^{-6}$
Pol 60	1	619 ± 26	460 ± 44	$2.01\ 10^{-5} \pm 1.00\ 10^{-5}$
Mean Value (N=22)				**$2.45\ 10^{-5} \pm 2.42\ 10^{-6}$**
Bronchial-MucilAir™				
Bron 009	8	371 ± 72	310 ± 23	$2.26\ 10^{-5} \pm 1.12\ 10^{-5}$
Bron 012	8	182 ± 0	198 ± 0	$1.82\ 10^{-5} \pm 8.96\ 10^{-6}$
Bron 014	6	236 ± 91	233 ± 40	$0.89\ 10^{-5} \pm 2.71\ 10^{-7}$
Bron 015	5	152 ± 23	191 ± 14	$0.93\ 10^{-5} \pm 8.26\ 10^{-7}$
Bron 016	3	480 ± 49	406 ± 107	$1.26\ 10^{-5} \pm 3.54\ 10^{-6}$
Mean Value (N=10)				**$1.43\ 10^{-5} \pm 2.83\ 10^{-6}$**
TOTAL Nasal-MucilAir™ and Bronchial-MucilAir™				
Mean Value (N=32)				**$2.13\ 10^{-5} \pm 2.05\ 10^{-6}$**

Nasal and bronchial transport of salicylic acid (1 mM; pH 7) has been studied on several batches of MucilAir™ with ages of cultures ranging from 1 to 13 months. No significant differences are observed, suggesting that nasal version (easier to obtain) could be a good surrogate to the bronchial epithelium for permeability studies.

Between-laboratory reproducibility



Transferability of the standard operating procedure was evaluated using 6 chemicals (propranolol, atenolol, nicotine, cadmium-chloride, cobalt-chloride and ammonium-hexachloroplatinate) in two independent laboratories.
Good reproducibility between-laboratory was observed,

Conclusions

1: SOP allowing ranking of relative permeability based on MucilAir™ is available.
2: Good within-laboratory reproducibility, transferability and between-laboratory reproducibility was observed.
3: Minor differences are observed between nasal and bronchial transport.
4: MucilAir™-based assay represents a promising tool to evaluate respiratory absorption giving input parameters for PBTK modelling.

Acknowledgements

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