In-vitro investigations of nicotine permeation in airway epithelial Calu-3 cells

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BACKGROUND

To better understand the systemic uptake of inhaled nicotine through the upper airways, we need to estimate values of permeation coefficients that can then be employed into more complex physiologically based pharmacokinetic models (PBPK). For this purpose, nicotine transport was analyzed experimentally in cultured human airway epithelial cells (Calu-3), as well as theoretically applying a simple pharmacokinetic model.

MATERIAL AND METHODS

Nicotine transport was investigated in Calu-3 cells cultured on membrane inserts under submerged conditions (Fig. 1). Nicotine was dissolved in culture medium and added to the apical compartment at concentrations of 10, 30, 100, 300, 1000 and 1500 µg/ml. The upper concentration was based on the assumption of a maximum amount of nicotine of 1.5 mg deposited in the airway generations 1 - 15 with an estimated lung lining liquid volume of 1 ml, while the lower values have been selected to cover a wide range of concentration to address potential concentration-dependent effects. The amount of nicotine transferred to the receiver compartment was quantified at four time-points (15, 30, 45 and 60 min) under steady-state conditions.

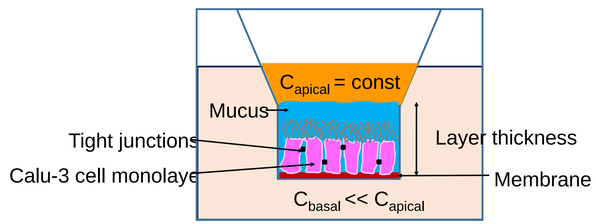


Fig.1: Schematic of Calu-3 cell line model for determination of nicotine transfer rate in the upper airway

Apparent permeability coefficients, P_{app}, were calculated according to the following equation:

$$P_{app} = \frac{\Delta m}{\Delta t * A * c_{0,apical}} \left[\frac{cm}{s}\right]$$

P_{app} (apparent permeability), Δm (mass transferred), Δt (transfer time), A (area), $C_{0,\,apical}$ (initial concentration in apical side)

A simple pharmacokinetic (PK) model, using the permeability coefficients obtained in-vitro as input parameters, was developed to estimate systemic nicotine uptake kinetics. The model has been developed for absorption in the upper airways and in the total lung and is comprised of three compartments: lung lining liquid layer, tissue (epithelium, interstitium, endothelium) and capillaries (Fig. 2). Morphological and physiological information was collected from literature.

Compartment 1: Lung lining fluid layer
Compartment 2: Epithelium, Interstitium, Endothelium
Compartment 3: Capillaries

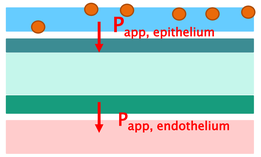


Fig.2: Schematic of 3-compartment model developed for investigation of systemic nicotine uptake kinetics

RESULTS

Calu-3 cells showed a good barrier formation before and after the experiments with transepithelial electrical resistance (TEER) values ranging from 558 to 477 Ω/cm². The apparent permeability coefficients, P_{app}, were largely independent of the donor concentration and were in the range of (1.2 – 1.5)x10^{-5} cm/s for the bronchial cell layer (Fig. 3).

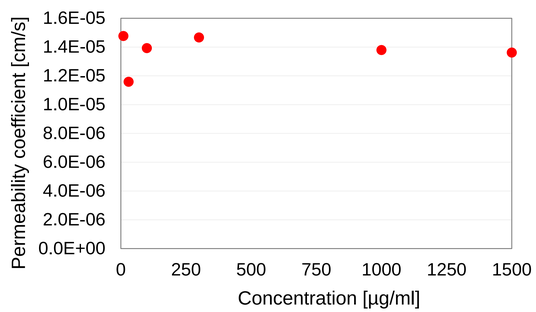
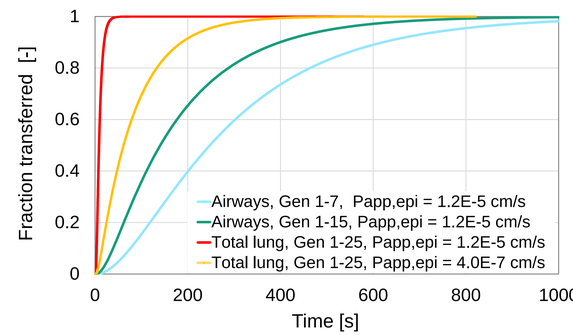


Fig.3: Apparent permeability coefficient as function of nicotine concentration, as determined in the Calu-3 cell model

The PK-model has shown that the absorption half-life is about thirty times larger for transfer through the upper airways only compared to absorption from the total lung including pulmonary region (250 s vs. 8 s) for a permeability coefficient of 1.2x10^{-5} cm/s (Fig. 4).



$P_{app,epi}$ = apparent permeability in epithelium layer

Fig.4: Nicotine absorption in different lung regions and for different epithelial permeability coefficients as calculated with the PK model

CONCLUSION

According to the experimental and theoretical investigations carried out, systemic uptake of nicotine in the different regions of the respiratory tract is assumed to be greatly affected by the total airway and capillary surface area in the relevant region of respiratory tract. This effect might even outweigh potentially reduced permeabilities for some substances observed for the alveolar region compared to the airways.

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DISCLOSURE

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