Advanced *In Silico* Modeling of the Performance of Inhalation Products

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Summary

In vivo C_{max} values and pulmonary absorption of budesonide delivered by Turbuhaler® were predicted with GastroPlus™ software, using either default pulmonary permeability data or *in vitro* experimental one. The experimental permeability data were gained using Calu-3 cells cultured on polycarbonate membranes (transwells) in submersion (SC) or under air-liquid interface (AIC) conditions to produce mucin. Calu-3 cells in AIC showed lower transepithelial electrical resistance (TEER) and higher permeability to budesonide than cells in SC. The produced mucin in AIC cell cultures did not reduce the transport rate of budesonide.
The experimental permeability data were lower than the calculated pulmonary permeability values predicted by the software. The software was able to predict *in vivo* performance reasonably well and the use of experimental permeability data further improved this prediction.
This work shows the supportive impact of combining advanced *in vitro* and *in silico* methods on the efficient modeling of pharmaceutical products *in vivo* performance. Development of such strong tools is the requirement for promoting safe and fast drug development.

Introduction

Development of patient-centric strategies for the improvement of medication adherence is strongly recommended by the World Health Organisation (WHO), U.S. Food and Drug Administration (FDA), and European Medicines Agency (EMA). Paving the way for safe and fast drug development is one of the key activities for the growth of such strategies. One of the strong supportive tools is predictive *in silico* modeling of active pharmaceutical ingredients (API) plasma concentration after medication using certain formulation and routes of administration. The pulmonary route of administration is well-known to be a non-invasive one for local and systematically acting drugs, providing advantages such as high speed of action, low first pass metabolism, absence of pH changes and of interfering factors (*e.g*. food).

Taking budesonide as an example, the aim of this work was i) to evaluate the efficiency of *in silico* modelling for the prediction of budesonide plasma concentration after pulmonary application, ii) to compare the impact of using own *in vitro* permeability data with default one on the prediction, iii) to compare a different way for treatment the cell culture, which leads to production of mucus, with the conventional method and investigate the impact of mucus on the cell permeability and in turn on *the in silico* prediction of budesonide plasma concentration.

Budesonide is a glucocorticoid, mainly used for the treatment of asthma and chronic obstructive pulmonary disease (COPD). The API is lipophilic with high first-pass metabolism both in the gut mucosa and the liver. We determined the *in vitro* permeability of budesonide using Calu-3 cell cultures, which derive from respiratory cells and produce mucus when cultured in a specific way. Mucus production plays a role for respiratory exposure because it may decrease the transport of some compounds across the epithelial monolayer [1, 2]
GastroPlus™ with Additional Dosage Routes Module™ was used for the simulation of the lung deposition, plasma concentration and the percentage of pulmonary absorption. The determined *in vitro* permeability was used as input parameter for the simulations, the results compared with those using the default permeability generated by the software.

Experimental methods

Material:

Budesonide and chemicals for analytical methods were purchased from Sigma-Aldrich (Austria). Calu-3 cells were obtained from the American Type Culture Collection (ATCC® HTB-55™). Chemicals for the cell culture were provided from Merck International (Austria).

Methods:

Cell culture: Cultured Human Airway Epithelial (Calu-3) cells were cultured on polycarbonate membranes (transwells) in submersion (SC) or at an air-liquid interface (AIC) and exposed to budesonide dissolved in Krebs-Ringer buffer). While Calu-3 cells in SC lack mucus production, they show mucus production in AIC. Mucin 5AC is a mucin produced by bronchial epithelial cells and has been detected by immunocytochemical staining (Figure 1).

Analytical method: HPLC-ESI-MS was used for the measurement of budesonide concentration using an Acquity UPLC® H-Class system (Waters) equipped with a photodiode array detector and a single quadrupole detector. Stationary phase was a Supersphер® 100 RP-18e column. Mobile phase was composed of (A) acetonitrile and (B) 10 mM ammonium acetate buffer pH 3.0 using the following gradient program: 2% A (0–0.5 min), 2-95% A (0.5-6.0 min), 2% A (6.1-10.0 min). At a flow rate of 0.6 mL/min, the retention time was of 7.1 min. Mass spectrometric detection was operated in single ion recording (SIR) mode using *m/z* 431.2. Capillary voltage was set to 0.5 kV and cone voltage to 25 V.

Simulation: GastroPlus™ v.7.0 (Simulations Plus), Additional Dosage Routes Module™ (ADRM) was used for the *in silico* modeling and simulation. The general physicochemical and biopharmaceutical parameters of budesonide for modeling were estimated using ADMET Predictor® (Simulations Plus). Physicochemical characterization of the formulation and *in vivo* data from volunteers were obtained from the literature [3, 4].

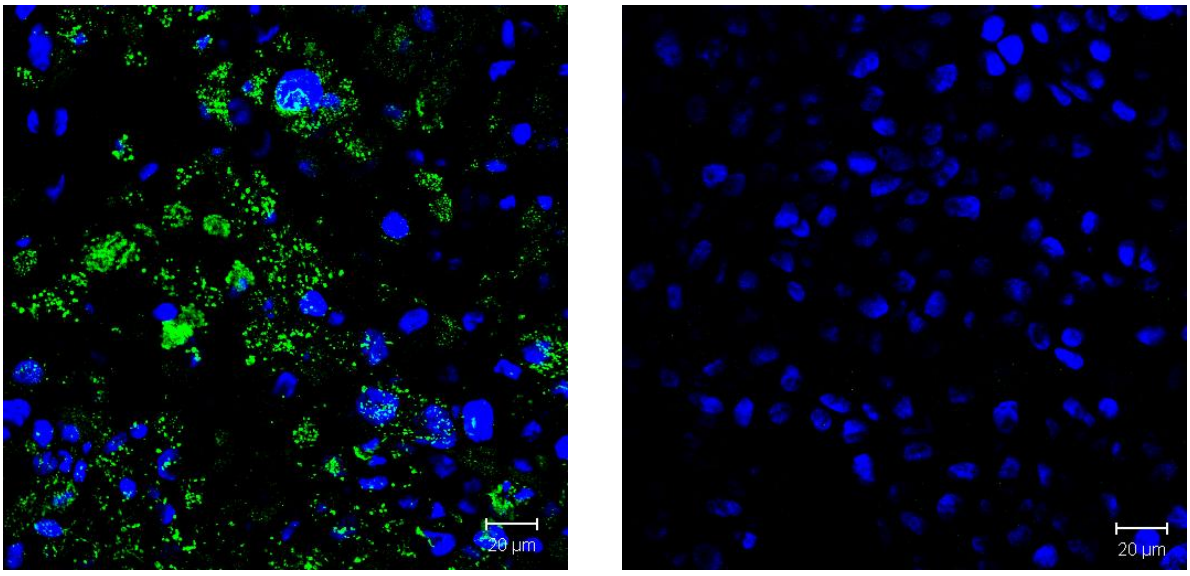

Figure 1 – Staining of Calu-3 cells with Anti-mucin 5AC Antibody (green) in AIC (left) and SC (right) Nuclei are counterstained with Hoechst 33342 (blue)

Results and Discussion

Calu-3 cells in AIC showed lower transepithelial electrical resistance (TEER) than cells in SC (Figure 2). The lower TEER value of cells in AIC has also been reported by other groups [5]. Figure 3 shows the P_{app} values of budesonide after application in SC and AIC cultures, showing 1.36 folds higher cell permeability in AIC, comparing to SC. The data shows that the produced mucus by cells cultured in AIC did not reduce the transport rate of budesonide.

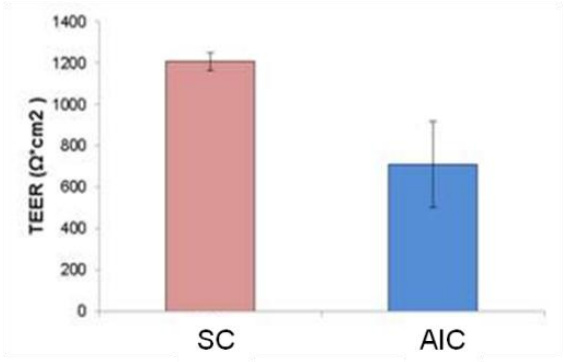

Figure 2 – TEER Values Measured in Calu-3 cells in SC and AIC Culture

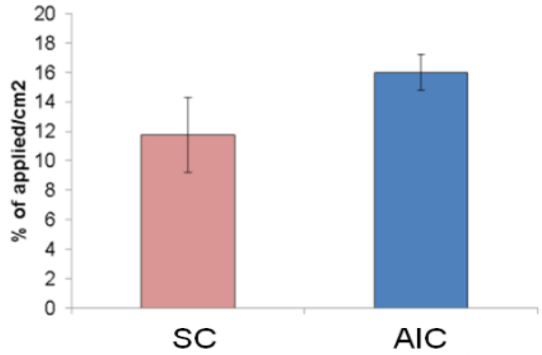

Figure 3 - Transport Rates (% of applied) of Budesonide by Application in SC and in AIC

The obtained permeability values were used as input parameters in GastroPlus™ for the prediction of budesonide C_{max} after application as dry powder inhaler (DPI). Using the gathered P_{app} values from AIC and SC cultures, the predicted values for C_{max} were comparable (Figure 4).

It is observed from Figure 4 that these predicted C_{max} values were closer to the reported *in viv*o C_{max} of 1.5 ng/mL and very close to the upper limit of reported 2.4 ng/mL. The predicted C_{max} based on the default permeability data of software was 3.12 ng/mL and 2-folds higher than the *in vivo* data. This is due to the higher P_{app} value calculated by the software compared to the P_{app} values calculated using SC and AIC cultures. The higher calculated value by the software can be explained by the fact that the P_{app} values are based on a data set of 16 hydrophilic APIs, and therefore it may not be ideal for the evaluation of hydrophobic APIs such as budesonide.

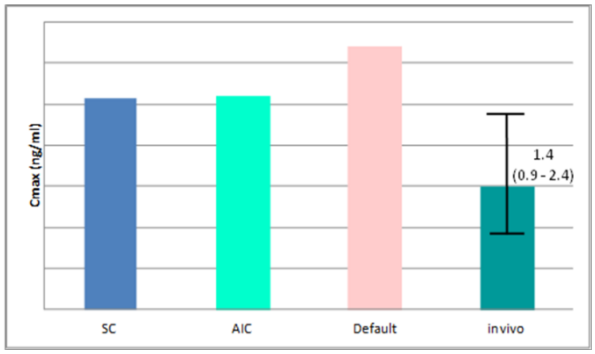

Figure 4- C_{max} of Budesonide Estimated with GastroPlus™ using Default Setting and P_{app} Values Obtained with the SC and AIC Cultures Compared to the *in vivo* Data

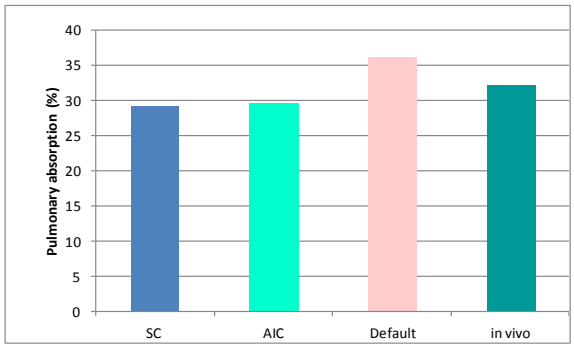

Figure 5- Predicted Pulmonary Absorption of Budesonide using Experimental Permeability Data and Default Values

Figure 5 shows the predicted pulmonary absorption of budesonide after application by Turbuhaler [3]. Using the experimental P_{app} values obtained from SC and AIC cultures, the predicted pulmonary absorptions were 29% and 30%, respectively. The slightly higher value for AIC is related to the higher P_{app} value. These predicted pulmonary absorptions were in very good agreement with the reported *in vivo* pulmonary absorption of 32% [3].

Conclusion

In this work we addressed the question to which extent different conditions in cell culture would affect the production of mucus and the permeability of budesonide. The data showed that the switch from submersed (SC) to air-liquid interface (AIC) culture stimulated the production of mucus, but did not reduce the permeability of budesonide. Using the *in vitro* permeability data for the simulation of budesonide plasma concentration resulted in the better fitting of predicted C_{max} with the *in vitro* C_{max}, as compared with the prediction using the default permeability data. Thus, it can be concluded. This work shows the supportive impact of combining advanced *in vitro* and *in silico* methods on the efficient modeling of pharmaceutical products *in vivo* performance.

References

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