HUMAN 3D AIRWAY MODELS TO PREDICT *IN VIVO* BIOAVAILABILITY UPON INHALATION

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OBJECTIVE

Human 3D airway models are promising models for safety and efficacy evaluation of compounds targeting the airways, because the models are fully differentiated and functional (incl. metabolic activity, mucus production and ciliary beating) and because they are cultured at an air-liquid interface allowing relevant exposure. It is anticipated that these models may predict a more realistic bioavailability of inhaled compounds instead of using a 100% uptake as default. To demonstrate this, we assessed absorption of several compounds by the MucilAir™ human 3D brionchial model (EpiThelix Sàrl) in models from healthy tissue and from asthmatic tissue (Figure 1).

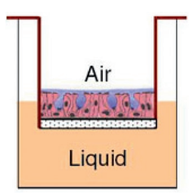
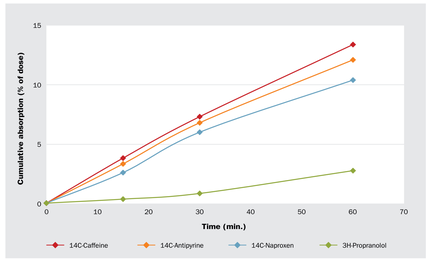
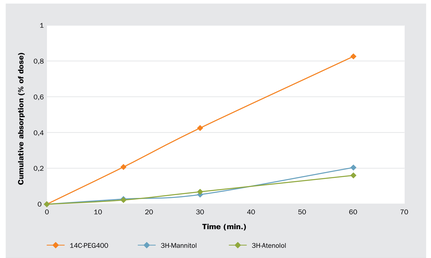


FIGURE 1
Schematic representation of the MucilAirTM model.

ABSORPTION IN HEALTHY TISSUE

3D inserts were exposed for up to 4 hours to radiolabeled test substances via droplets on the tissue surface. Absorption was determined using liquid scintillation counting of the receptor fluid (basal side), cellular fraction and (apical) washing fluid, including the mucus layer (Figures 2a, b and 3).





FIGURES 2a and b
Concentration of in media of the test substance over time. Results show that the model is able to differentiate between compounds utilizing various transport mechanisms, including passive uptake transcellularly (e.g. caffeine, fig 2a) or paracellularly (e.g. mannitol, fig 2b).

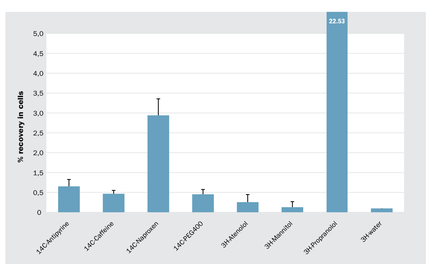
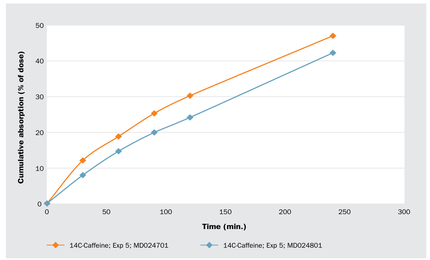


FIGURE 3
Cellular uptake of test substances over time.

ABSORPTION IN ASTHMATIC TISSUE

Impaired respiratory tissue is expected to have an altered uptake of substances, which in part may explain the strong response of asthmatics to inhalation of mannitol. Therefore, uptake of mannitol, caffeine and insulin was also assessed with inserts obtained from asthmatic patients (Figure 4).



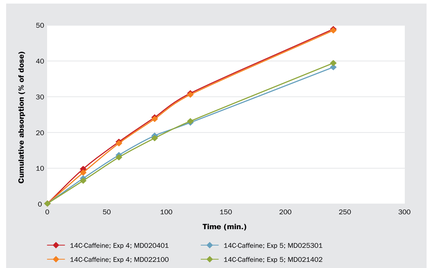
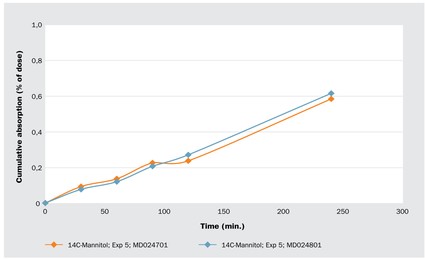


FIGURE 4a
Absorption of mannitol in healthy and asthmatic tissue. Increased uptake of mannitol was found compared to healthy donors, but the response depended heavily on the donor. This suggests a considerable interindividual variation in diseased tissues, which is considered a realistic reflection of the interindividual variation in humans.



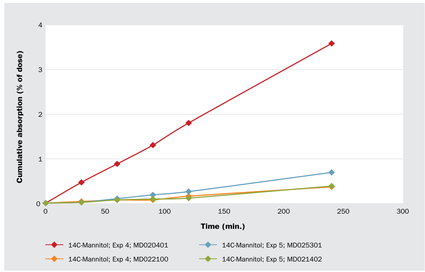
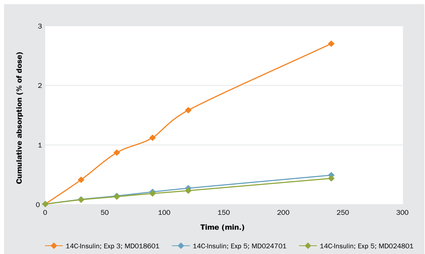


FIGURE 4b
Absorption of caffeine in healthy and asthmatic tissue. Uptake of caffeine was comparable between healthy and asthmatic tissue. Some interindividual varation was observed.



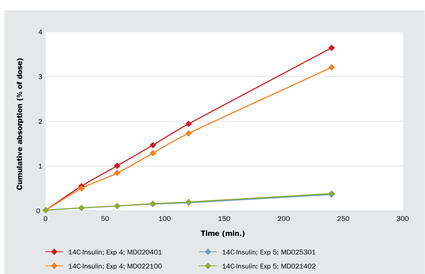


FIGURE 4c
Absorption of insulin in healthy and asthmatic tissue. Uptake of insulin was comparable between healthy and asthmatic tissues, but a considerable inter-individual variation or variation between experiments or both was found.

CONCLUSION

Results obtained so far indicate that MucilAir™ is a robust model for the evaluation of *in vitro* absorption, because of the ability to differentiate between compounds utilizing various transport mechanisms, including passive uptake transcellularly and paracellularly.

Generally the results obtained with MucilAir™ are considered reproducible, though absolute values can be different between experiments and donors. Larger donor variability is expected with compounds utilizing primarily a trans-cellular transport mechanism (e.g. mannitol) compared to a paracellular transport mechanism (e.g. caffeine). Insulin may use a transcellular transport mechanism, but also other transport mechanisms.

OTHER AND FUTURE PLANS

In future, we will further assess the applicability of these models for exposure via the air (dynamic test atmosphere) and compare the results with *in vivo* inhalation data. Additionally, we explore the use of these models for other (toxicological) endpoints, including:

- Acute and local toxicity
- Genotoxicity (micronucleus test, COMET assay)
- Inflammation and oxidative stress

Ultimately, these models may be useful in the safety evaluation of compounds for which the airways are the primary route of exposure.

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