EVALUATION OF A HUMAN IN VITRO 3D RESPIRATORY EPITHELIUM MODEL IN BIOAVAILABILITY AND SAFETY ASSESSMENT OF PHARMACEUTICAL AND CHEMICAL COMPOUNDS

Joost Vos[1];
Yvonne Staal[1];
Jos van Triel[1];
Frédérique van Acker[1];
Astrid Reus[1];
Evert Duistermaat[1];
Wilfred Maas[1];
Mariska Gröllers-Mulderij[2];
Maaike Steenhof[2];
Rob Stierum[2];
Linette Pellis[2];
Eugene van Someren[2];
Eric Schoen[2];
Frieke Kuper[2];
Ingeborg Kooter[2].

1 TNO Triskelion B.V., P.O.BOX 844, 3700 AV Zeist, The Netherlands
2 TNO, P.O.BOX 360, 3700 AJ Zeist, The Netherlands

For further information: joost.vos@tno.triskelion.nl

INTRODUCTION:

The lungs are increasingly used as administration route for pharmaceuticals and are an important entry route for inhalable chemicals. Laboratory animals are mostly used in safety and efficacy evaluation of inhalable test compounds, although animals may not necessarily reflect human physiology or human disease *(i.e. allergic asthma and COPD)*. Hence, there is a need for predictive models mimicking human physiology. Progress has been made in developing three dimensional (3D) models. MucilAir™ is an *in vitro* model consisting of human basal, goblet and ciliated cells cultured at an air-liquid interface featuring a fully differentiated and functional respiratory epithelium. The model displays *in vivo* characteristics including tight junctions, metabolic activity, mucus production and beating cilia. Such 3D models may find applications in the predictive *in vitro* safety and efficacy evaluation of pharmaceutical or chemical test compounds.

OBJECTIVE

To evaluate the MucilAir™ 3D respiratory epithelium model compared to non-primary *in vitro* models for assessing *(i) bioavailability* of pharmaceutical compounds and *(ii) safety* of nanoparticles.

METHODS:

For bioavailability assessment MucilAir™ inserts ***(Figure 1)*** were exposed to radio-labelled ^{14}C-Caffeine, ^{14}C-Antipyrine, ^{14}C-Naproxen, ^{3}H-Atenolol and ^{14}C-PEG400 via the apical surface.

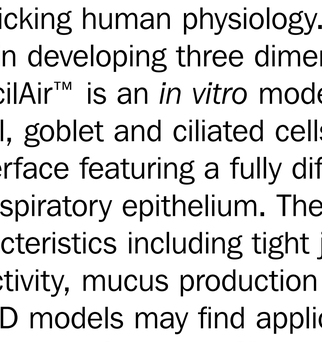

Figure 1 Schematic representation of MucilAir™ Air-Liquid-Interface culture – *Cells are cultured on a membrane. The upper side of the culture is air exposed while the basal side is surrounded by culture medium.*

Bioavailability of radio-labelled compounds was assessed over a period of 1 hour by means of liquid scintillation counting of the apical dose solution, epithelial cells and basolateral culture medium.

To assess its application in safety testing, MucilAir™ inserts were exposed to atmospheres containing cerium oxide nanoparticles. Parameters assessed included tissue integrity *(transepithelial electric resistance)*, cell membrane integrity *(lactate dehydrogenase [LDH] leakage)*, oxidative stress *(HO-1; heme oxygenase protein activity)*, genotoxicity *(Comet assay)* and Illumina gene array expression analysis.

BIOAVAILABILITY

The bioavailability of ^{14}C-caffeine, ^{14}C-Antipyrine, ^{14}C-Naproxen increased linearly to up to 13%, whereas ^{3}H-Atenolol and ^{14}C-PEG400 were hardly absorbed ***(Figure 2)***. The 3D respiratory epithelium model allowed to discriminate between easily absorbed compounds *(Antipyrine, Caffeine and Naproxen)* and difficult to absorb molecules *(Atenolol, PEG400)*. We also assessed ^{14}C-insulin, which showed intermediate absorption and bioavailability *(data not shown)*.

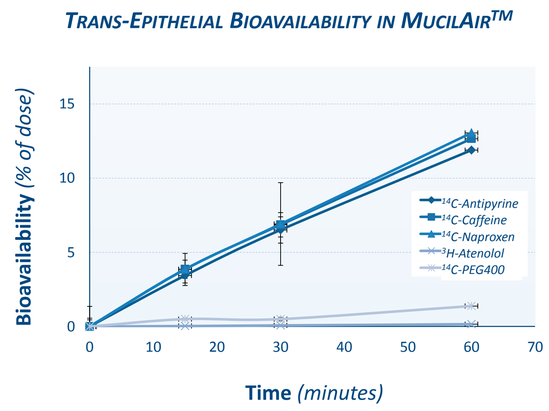

Figure 2 Bioavailability of radio-labelled test compounds in receptor fluid – *Cumulative bioavailability of test compounds in basolateral culture medium in time ± standard deviation.*

SAFETY EVALUATION OF AEROSOLIZED NANO-PARTICLES

To evaluate the MucilAir™ model for in vitro air exposure safety testing, we placed the cell-containing inserts in Vitrocell's modules. MucilAir™ was compared to the non-primary cell lines A549 and BEAS2B cultured in monolayers. After exposure to aerosols of cerium oxide nanoparticles the MucilAir™ model showed an increased anti-oxidative HO-1 activity. Such response was absent non-primary cell lines A549 and BEAS2B ***(Figure 3)***.

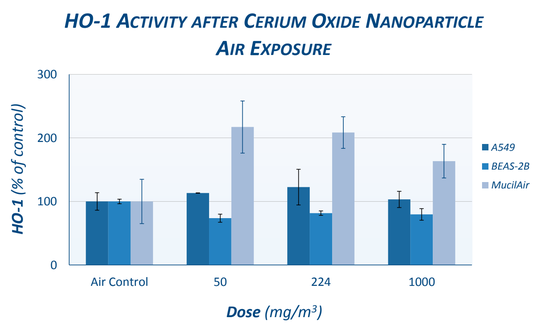

Figure 3 Heme Oxygenase 1 Activity – *Activation of the oxidative stress pathway in MucilAir™ cells as determined by the increased HO-1 activity.*

By contrast, genotoxicity was observed in A549 and BEAS2B cells after nanoparticle exposure ***(Figure 4)***, whereas genotoxicity was absent in the MucilAir™ model.

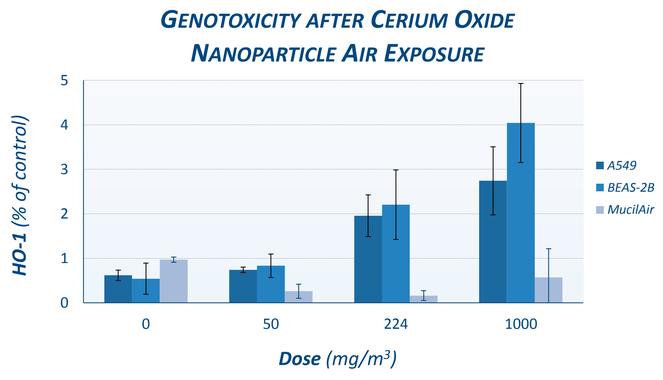

Figure 4 Comet Assay for Genotoxicity – *No DNA damage was observed in MucilAir™ after cerium oxide nanoparticle exposure, whereas a genotoxic effect was observed in A549 and BEAS-2B cell lines.*

These in vitro data correspond to our previous findings that aerosols of nanoparticle were non-genotoxic in rats *in vivo*[a] and with recent publication showing that that cerium oxide nanoparticles were non-genotoxic to human lens epithelial cells *in vitro*[b]. Gene expression profiling revealed a clear distinction between 3D primary MucilAir™ and 2D non-primary A549 and BEAS2B models ***(Figure 5)***.
This shows that the cell models used have clear distinct characteristics.

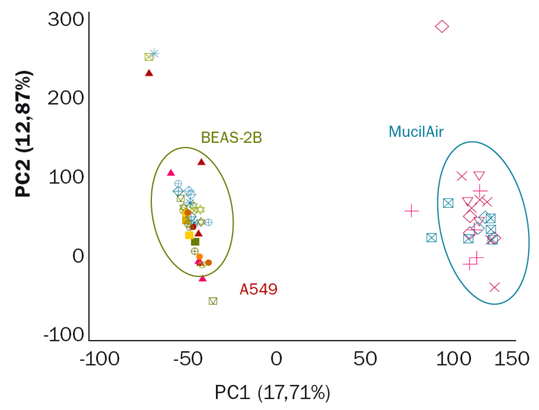

Figure 5 Comparative Gene Expression Analysis – *Gene expression in MucilAir™ cells is clustered separately from A549 and BEAS-2B. Changes in gene expression patterns may explain the differences observed in response to the test compounds.*

CONCLUSIONS

Primary cells appeared more resistant towards experimental air flow compared to non-primary cells, most likely due to the presence of tight junctions, mucus and beating cilia ***(Figure 6)***.

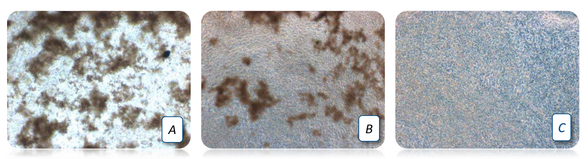

Figure 6 Analysis of Ciliary Clearance of Cerium Oxide Nanoparticles on MucilAir – *Microscopic images of MucilAir immediately after apical surface administration of cerium oxide nanoparticle (A), 1 hour after exposure and (C) 24 hours after exposure. Active ciliary beating removes nanoparticles from the center surface towards the outside rim of the insert.*

Our results show that the MucilAir™ respiratory epithelium model allows the assessment of bioavailability and safety of pharmaceutical and chemical compounds with the airways and lungs as intended route of delivery. We optimized the use of Vitrocell modules for air exposure application of test compounds onto the cells mimicking in vivo conditions. The battery of read-out parameters included, but is not limited to *(i) oxidative stress, (ii) release of inflammatory markers, (iii) cytotoxicity, (iv) genotoxicity, (v) large-scale gene expression analysis and (vi) cellular transporters.* This 3D model of respiratory epithelium may find applications in predictive screening of bioavailability and safety of pharmaceuticals and chemicals for which the airways are the primary route of exposure.

a Pulmonary toxicity of cerium dioxide particles is modulated by size and coating: inhalation studies with nano-, aggregated nano- and micron-scale particles and particles with different surface chemistry. Staal YCM, Oral presentation at the 2010 Annual Meeting of the Society of Toxicology, Salt Lake City, Utah.

b Nanoceria have no genotoxic effect on human lens epithelial cells. Pierscionek et al., Nanotechnology 2010 Jan 22;21(3):035102.

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