Human In Vitro Models for Respiratory Toxicology: Evaluation of Goblet Cell Hyperplasia, Mucus Production, and Ciliary Beat Assays

S. Frenzel1, M. Aragon2, J. Hoeng3, S. Ito4, S. Ishikawa5, J. Budde6, A. Mainone7, P. Hayden8, W. Fields9, B. Keyser10, L. Haswell11, D. Azzopardi12 and H. Behrings3

1Philipp Morris International, Neuchatel, Switzerland; 2IVS, Inc., Gaithersburg, MD; 3Japan Tobacco, Yokohama, Japan; 4ILT-Reemtsma Zigarettenfabriken GmbH, Hamburg, Germany; 5MatTek, Inc., Ashland, MA; 6FAI Research Laboratory, Winston-Salem NC and 7British American Tobacco R&D, Southampton, United Kingdom.

ABSTRACT

Robust non-animal models and assays for pulmonary toxicology are required to make competent product development and risk assessments for new materials requiring toxicity testing. The effects of tobacco smoke in vitro assays (goblet cell hyperplasia (GCH), cilia beat frequency (CBF), and MUC5AC quantification) were evaluated for performance and reproducibility. To assess these assays, 6 laboratories contributed model data using a common protocol utilizing IL-13 as an inducer of airway mucous-relevant tissue changes. MatTek Epithil™ and Ephelitic MucilAir™ models were used to validate endpoints using histology for GCH, software-based applications, Ciba FA and SAF, for CBF, and ELISA assay for MUC5AC. Continuous 10 ng/mL IL-13 (GCH), IL-13 (GCH) or no IL-13 (10 pmol) peptide (CBF) exposures prior to day 7 and 14 time points were included as positive controls. Quality control endpoints (in IL-13 peptide content and trans-epithelial electrical resistance) were also evaluated. Multi-fold increases (ranging from 2.6 to 33-fold and 1.5 to 238-fold) in MUC5AC expression and CBF over untreated controls were achieved. Significant increases in goblet cell hyperplasia and IL-13 induced a significant decrease as expected. However, the MUC5AC data did not yield consistent results when between-laboratory comparisons were performed and analyzed. These results suggest these non-animal test systems may provide additional validations that could facilitate long-term testing. These data, utilized in a prognostic manner, can be used in vitro assays that have the potential to be included in a Reduced Risk Product assessment framework.

MATERIALS & METHODS

INTRODUCTION

The Family Smoking Prevention and Tobacco Control Act of 2009 established the FDA Center for Tobacco Products (CTP) and gave the agency regulatory authority over the marketing, manufacture and distribution of tobacco products, including those termed "modified risk". On Oct. 22, 2014, CTP released the second version of "Assessment of in-vitro COPD Models for Tobacco Regulatory Science" to bring together stakeholders representing regulatory agencies, academia, industry and animal protection to address the research priorities articulated by the FDA CTP. Specific topics were covered to assess the status of current in-vitro technologies as they are applied to understand the adverse pulmonary events resulting from tobacco product exposure. The four topics covered were: 1) Information and Ossification Stress, 2) Ciliary Dysfunction and Transport, 3) Goblet Cell Hyperplasia and Mucous Production and 4) Parenchymal/bronchial Tissue Construction and Remodeling. Breakout session discussions were held for three of the four core topics and were intended for consolidating current views on assay endpoints, test systems, in-vitro technologies that should be considered for standards, and identifying areas that require additional research and/or development. Conclusions drawn from the breakout groups resulted in these in-vitro models in the "Mucous Goblet Cell Hyperplasia (GCH)" and "Ciliary beat frequency(CBF)" being identified that merit further exploration.

RESULTS

Test System: Human Reconstructed Airway (RhB) grown at the Air-Liquid Interface (ALI) (n>1 per treatment and time-point). Reference materials: 1. IL-13 induction of mucous producing goblet cells for 14 days.
2. Procarcin (8222 adenocarcinoma) for CBF stimulation for 10 min.

Procedure: All laboratories coordinated orders with one or both tissue manufacturer to receive rhinopharynx from the same donor cells. Cells were obtained from rhinopharynx in MatTek's Goblet cell hyperplasia kit (D7) and stored at −140°C until shipped to each lab. Upon receipt of cells, an aliquot was split in two sets and frozen to assess for IL-13 treatment-induced goblet cell hyperplasia (GCH). Each set was then thawed and assessed. These results suggest these non-animal test systems may provide additional validations that could facilitate long-term testing. These data, utilized in a prognostic manner, can be used in vitro assays that have the potential to be included in a Reduced Risk Product assessment framework.

REFERENCES


MATERIALS & METHODS

CONCLUSIONS

The Inter-laboratory comparison of assay data using a common protocol identified strengths and weaknesses of all three endpoints.

1. GCH: Both commercially available tissues were responsive to IL-13 induction of goblet cell hyperplasia. Induction of GCH was variable across labs and across orogen, the latter was possibly due to donor cell properties.

2. MUC5AC: Deep sequencing technologies were utilized to address reproducibility. The raw counts for the rhinopharynx samples in all the laboratories yielded unrepeatable results. The use of Elisa to quantify MUC5AC from rhinopharynx samples was not reliable, including storage conditions of samples prior to measurement.

3. CBF: The in-vitro models were responsive to IL-13 exposure up to 14 days, however, the SAF software appeared to perform consistently. RhB displayed different levels at % Active Cilia (mobile cilia).

Additional testing of technical factors related to assay endpoints will allow optimisation of testing procedures. Elements include the number of CBF fields required per intact, Elisa performance for rhinopharynx sampling, impact of rhinopharynx acclimation length, etc.

ACKNOWLEDGMENTS

The authors thank all the following groups for their help, contributions and support: MatTek; Elisa software developers; through the Interlab study (including the abstract and poster review by Dr. Mike Oldham) and RAI, the Tobacco Use Prevention Endpoints Working Group, (MUC5AC ELISA) for MUC5AC staining (Goblet) Cells (% of all cells).