Human In Vitro Models for Respiratory Toxicology: Evaluation of Goblet Cell Hyperplasia, Mucus Production, and Ciliary Beating Assays
S. Frenzel1, M. Aragon2, J. Hoeng1, S. Ito3, S. Ishikawa4, J. Budde5, A. Mainone6, P. Hayden7, W. Fields8, B. Keyser9, L. Haskell10, D. Azzopardi11 and H. Behrsing2
1Philipp Morris International, Neuchâtel, Switzerland; 2IIVS, Inc., Gaithersburg, MD; 3Japan Tobacco, Yokohama, Japan; 4ITL-Reemtsma Cigarettenfabriken GmbH, Hamburg, Germany; 5MatTek, Inc., Ashland, MA; 6RAI Services, Cambridge, United Kingdom; 7British American Tobacco

ABSTRACT

Toxicological analyses that require readouts of goblet cell hypertrophy (e.g., IL-13 or EpiAirway™) for in vitro assays (goblet cell hyperplasia [GCH], IL-13 secretion [CBF], and MUC5AC quantification) were evaluated for performance and reproducibility. To assess these assays, 6 laboratories contributed data using a common protocol utilizing IL-13 as an inducer of airway mucin-related tissue changes. MatTek EpiAirway™ and Epithelium Mucilair™ (EM) models were used to evaluate endpoints using histology for GCH, software-based applications, Cita PA and SAIA, for CBF, and ELISA assay for MUC5AC. MatTek's Continuous 10 ng/mL; IL-13 (GCH); ELISA exposure; or one 10 µg/provocation (CBF) exposures prior to day 7 and time-point were included as positive controls. Quality control endpoints for GCH, IL-13 and SAIA (airway epithelium resistance) were also evaluated. Multi-fold increases (ranging from 2.6 to 33.0) and 1.5- to 238-fold in MUC5AC were found after IL-13 stimulation, with IL-13 stimulation inducing a significant increase, and IL-13 elicited a significant decrease as expected. However, the reliability of the MUC5AC assay did not yield consistent results when tissue mass was thawed and assayed. These results suggest these non-animal-test systems may provide accurate readouts of goblet cell hypertrophy and CBF, but require additional validation to improve reproducibility in multisite testing. A streamlined protocol using these controls will be applied toward additional testing of these assays, utilizing a pragmatic manner with data in vitro assays have the potential to be included in a Reduced Risk Product assessment framework.

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” on Day 10. On Day 7, mature human nasal epithelial cells were exposed to IL-13 (2 ng/mL) or control and examined on Day 14 for GCH and CBF. The authors found that Day 10 CBF was identified that merited further exploration.

RESULTS

Goblet Cell Hyperplasia

The IIVS and BAT assays were conducted in multiple labs in June 2015. A group of assay experts met to discuss test conditions for the three assays (IL13, EpiAirway™ and CBF) that were thought to pose potential challenges in the reproducibility of results. Three sets of pre-conditioned airway models of the human respiratory tract, participant labs included IIVS, Inc., British American Tobacco, R&D (BAT), and PMI ITL, and New York City (NYC) for Interlaboratory Testing (NCI). As per our guidelines, all labs were required to harmonize sample handling and analysis procedures.

The IIVS CBF assay was considered to be the most robust of the three assays evaluated in this study. The ELISA was performed in duplicate in each lab, and the results were consistent across labs and across vendors; the latter was possibly due to donor cell properties. In the IIVS ELISA data analysis, the IL-13 treatment group exhibited a greater magnitude of response, and statistical analysis revealed that the IL-13 treatment group was significantly different from the untreated group (p < 0.05). The R&D group also observed a trend towards increased CBF in the IL-13 treatment group compared to the untreated group (p = 0.066). The IL-13 treatment group also exhibited a greater magnitude of response compared to the untreated group in all laboratories except for the NYC lab, where no significant differences were observed. These results suggest that the IIVS ELISA assay is a robust and reliable method for assessing CBF in human airway models.

CONCLUSIONS

The IIVS ELISA assay was the most robust and reliable method for assessing CBF in human airway models. The IL-13 treatment group exhibited a greater magnitude of response compared to the untreated group in all laboratories except for the NYC lab, where no significant differences were observed. These results suggest that the IIVS ELISA assay is a robust and reliable method for assessing CBF in human airway models.

REFERENCES


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