Toxicity of cadmium chloride in a 28-day repeated exposure human bronchial epithelial cell model

Emma Bishop, Damien Breheny, Fiona Cunningham, Stacy Fiebelkorn, Debbie Dillon, Clive Meredith
British American Tobacco, Group Research and Development, Southampton, SO15 8TL, United Kingdom.
Correspondence: emma_bishop@bat.com

INTRODUCTION

Tobacco smoke contains over 5,600 constituents, some with well-established toxicological properties (1). To date, approximately 150 cigarette smoke constituents have been identified as ‘tobacco smoke toxicants’ with defined toxicological properties. This includes the heavy metal cadmium, which we have identified as a high priority for exposure research reduction on the basis of Margin of Exposure calculations and its inclusion in the WHO Study Group on Tobacco Product Regulation (TabReg) list for mandatory monitoring. Our proposed framework for risk assessment of toxicants combines both computational and in vitro experimental components. The Mode of Action (MOA) for cadmium is postulated to involve impaired lung function leading to increased mucus production, oedema and emphysema. Exposure to cadmium is further linked to non-squamous cell carcinoma via an oxidative stress mechanism. The aim of this study was to inform the MOA by determining cadmium’s mechanism of lung toxicity.

METHODS

Normal human bronchial epithelial cells (NHBEs) from three independent male Caucasian donors were differentiated in a 28-day basolateral exposure model with daily (Monday-Friday) cadmium chloride treatment (0-5µM). Control conditions of 5µg/ml cigarette smoke particulate matter (PM) and 5mg/ml interleukin 13 (IL-13) recombinant protein were also included in each experiment. PM and IL-13 are known to induce mucin expression in NHBE cells (3).

Cytotoxicity was measured by neutral red uptake at day 7, 21 and 28 along with membrane integrity as measured by trans epithelial electrical resistance (TEER), MUC5AC production, cell morphology, cilia beat frequency and 8-oxo-dG adduct formation were tested in day 28 cells and secreted protein expression were assessed throughout the period of cellular differentiation. Immunohistochemistry was further quantified using MetaMorph® software. 15 nuclei per slide were assessed for staining pixel intensity.

Data from three donors in three replicate experiments were averaged. Statistical significance was determined using a general linear model ANOVA.

RESULTS

Neutral red uptake was conducted to assess the cytotoxicity of cadmium chloride. At all time points (day 7, 21 and 28) none of the treatment groups were cytotoxic (less than 20% loss in cell viability) as shown in Figure 1A. At each time point the electrical resistance across the Transwell insert was also assessed, as a measure of membrane integrity. The data are shown in Figure 1B. Cadmium chloride induced a statistically significant reduction in membrane barrier function at Day 21 and 28 (p<0.001).

Cilia beat frequency was determined in day 28 differentiated samples. Data are shown in Figure 2. Statistical evaluation of the data showed that cadmium treatment has a significant effect on beat frequency. There was also an observed decrease in cilia coverage in these samples. IL-13 and PM controls did not have a significant effect on beat frequency.

Immunohistochemistry was also undertaken in day 28 samples. Mucin expression was determined by MUC5AC staining as shown in Figure 3A. PM and IL-13 had a significant effect on mucin production as well as cadmium chloride, the largest increase at 1.25µM. The presence of 8-hydroxy-2-deoxyguanosine (8-oxo-dG) lesions was also assessed by immunohistochemistry as shown in Figure 3B. PM exposure had a significantly positive effect on 8-oxo-dG lesions and cadmium chloride induced a dose-dependent increase in 8-oxo-dG lesions (p<0.001).

DISCUSSION

Under the concentrations tested, cadmium chloride exposure reduces the functionality of lung epithelial cells in vitro and induces a goblet cell hyperplasia-like phenotype. Oxidative DNA adduct formation may also play a role in cadmium-induced carcinogenesis.

CONCLUSION

These results support the plausibility of a number of key events in the postulated cancer and non-cancer MOAs for cadmium and add weight of evidence for its status as a high priority for exposure reduction research in tobacco smoke. 

REFERENCES