Popcorn lung: Diacetyl exposure of primary human bronchial epithelial cells using a relevant air-liquid setup

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Introduction

Diacetyl is used as butter flavorant in the microwave popcorn industry and is known to cause bronchiolitis obliterans in popcorn plant workers. This is a rare obstructive pulmonary disease, in which the airway epithelium is the primary target of injury. E-cigarette smoking is also a potential route of exposure, as diacetyl is a common constituent in e-cigarette flavorings.

Objectives

The major goal of this study was to evaluate the effects of diacetyl on primary human bronchial epithelial cells with regard to viability and monolayer integrity. The volatility of diacetyl requires specific attention regarding the exposure conditions. We thus developed a specific exposure setup for the exposure of air-liquid interface (ALI) cultures to the gas phase of this volatile compound based on the P.R.I.T.® ExpoCube® device (Ritter & Knebel 2014). This unique exposure device provides a highly efficient exposure situation by preventing contact between the test compound and the culture medium.

Materials and Methods

Primary human bronchial epithelial cells were either obtained from a commercial source (primary normal human bronchial/tracheal epithelial - NHBE, Lonza) or isolated from tumor-free resected lung tissue from four donors (PBEC). All cell models were allowed to differentiate into airway epithelium at ALI conditions. The test atmosphere was generated by conducting clean air over the surface of diacetyl inside a gas washing bottle at 25°C. The resulting atmosphere was diluted with clean air to achieve diacetyl concentrations of 102, 135, 277, 323 and 1834 ppm. Online analysis of diacetyl during exposure was done by FT-IR spectroscopy. Primary human bronchial epithelial cells were exposed for 1h once or repeatedly on three consecutive days. Cellular viability was measured by means of LDH-leakage and WST-test. In addition, monolayer integrity was checked by measuring the transepithelial electrical resistance (TEER).

Experimental setup

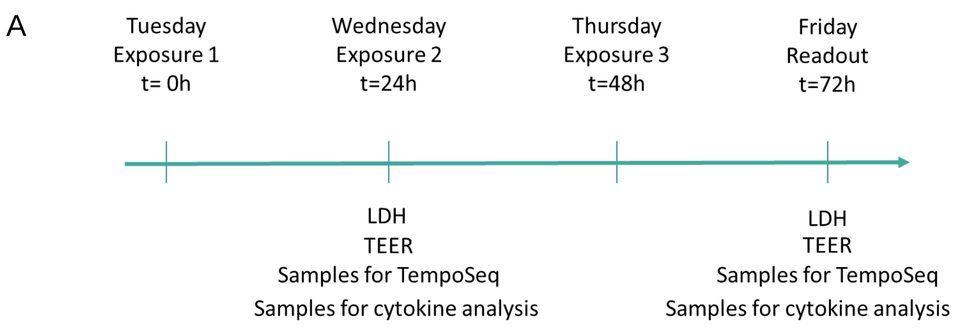

Figure 1: Scheme of the *experimental setup* for exposing primary human bronchial epithelial cells to volatile compounds using air-liquid interface conditions. A) exposure design and time points of the conducted readouts: single exposure for 1h on exposure day 1, readout 24h after exposure; repeated exposure for 1h on the consecutive exposure days 1, 2 and 3, readouts 72h after the start of exposure **B)** The system consists of three parts: A: Generation and transport of the test substance atmosphere (1-4) or the clean air (5) control respectively. B: The exposure unit for the target cells, grown under air-liquid interphase conditions (P.R.I.T.® ExpoCube® ;6). C: Analysing unit for testatmosphere (FT-IR Monitor); Position 4 represents the exhaust of excess testatmosphere.

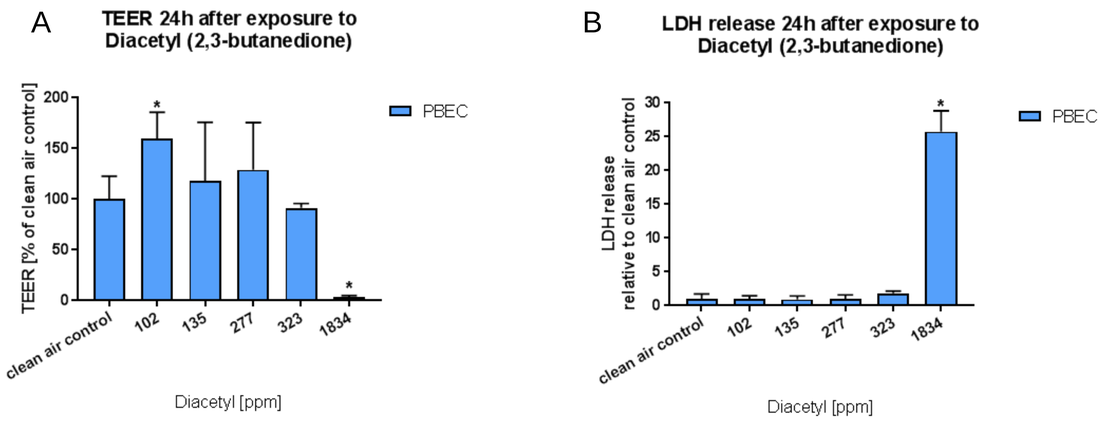

Figure 2. Measurement of transepithelial electrical resistance (TEER) values and LDH release 24h after exposure to diacetyl: Primary human bronchial epithelial cells isolated from four different donors (PBEC) were exposed for 1h to increasing concentrations of diacetyl. TEER values (A) and LDH release (B) were measured 24h after the single exposure. *p<0.05 versus clean air control by one-way ANOVA and Tukey's test.

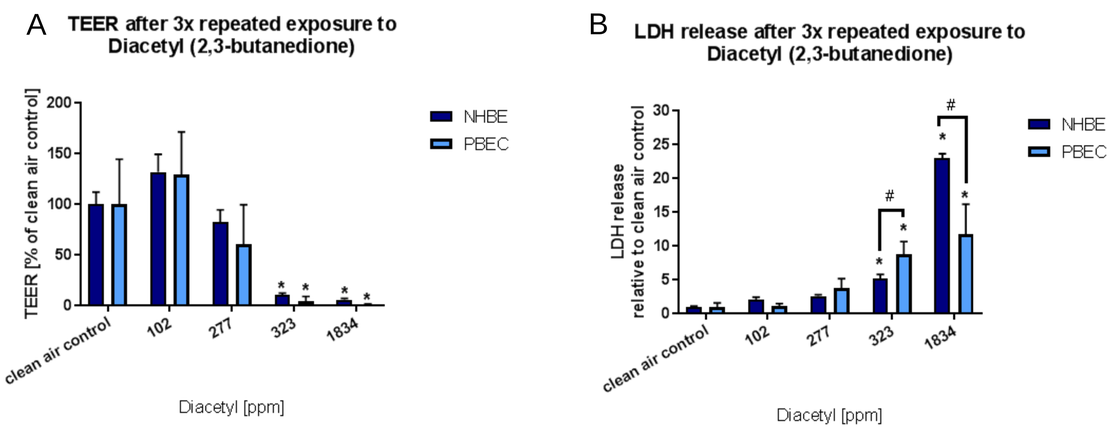

Figure 3. Measurement of transepithelial electrical resistance (TEER) values and LDH release after 3x repeated exposure to diacetyl: Primary human bronchial epithelial cells isolated from four different donors (PBEC) or provided from Lonza (NHBE) were exposed for 1h repeatedly on three consecutive days to increasing concentrations of diacetyl. TEER values (A) and LDH release (B) were measured 72h after the first exposure. *p<0.05 versus clean air control by one-way ANOVA and Tukey's test, #p<0.05 NHBE versus PBEC by two-way ANOVA followed by Tukey's multiple comparisons test.

Results

- Single exposure for 1h led to a complete loss of TEER and a significant increase in LDH leakage at the highest concentration (Fig. 2). After repeated 1h exposure for three days, TEER was significantly broken down at 323 ppm and a significant dose-dependent increase in LDH leakage was observed starting at 277 ppm (Fig. 3).
- Three times repeated exposure of primary human bronchial epithelial cells to diacetyl displays a clear lower observed adverse effect level (323 ppm) compared to one single exposure (1834ppm) (Fig. 2 and 3).
- TEER values measured after 3x repeated exposure to diacetyl did not show a statistically significant difference between primary human bronchial epithelial cells isolated from four different donors (PBEC) and provided from Lonza (NHBE). Considering the LDH leakage a significant difference between PBECs and NHBEs was observed after 3x repeated exposure to 323 ppm diacetyl (Fig. 3).

Conclusions

in vitro testing of volatile gases requires special technical requirements like the P.R.I.T.® ExpoCube® to provide correct quantitative statements and to avoid false positive or negative results. Under these conditions, diacetyl revealed a dose-dependent toxic effect on primary human lung cells.

Outlook

- ALI exposure of PBECs to structurally related compounds (α, β and γ diketones)
- Gene expression analysis of the whole transcriptome
- Cytokine analysis

References

Ritter, D., Knebel, J, "Investigations of the biological effects of airborne and inhalable substances by cell-based in vitro methods: fundamental improvements to the ALI concept", Advances in Toxicology Volume 2014, Article ID 185201,.

Acknowledgement

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