Effects of Short- and Long-Term Exposure to E-Cigarette Vapour

DZL – Deutsches Zentrum für Lungenforschung
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2019 Balzan Prize for Pathophysiology of Respiration: from Basic Sciences to the Bedside

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Administrating Institution: Deutsches Zentrum für Lungenforschung
Period: 2020-2025

Erika von Mutius is a pediatrician and allergologist at the Helmholtz Center in Munich, and at the Ludwig-Maximilians-University of Munich. Klaus F. Rabe is Medical Director and Managing Director at Grosshansdorf Hospital and W3 Chair in Internal Medicine/Pulmonology at the University of Kiel. Werner Seeger is Director and Scientific Member at the Max Planck Institute for Heart and Lung Research, Bad Neuheim. Tobias Welte was Professor of Pulmonary Medicine and Head of the Department of Pulmonary and Infectious Disease at Hannover University School of Medicine.

General Report

The winners of the Balzan Prize 2019 – Erika von Mutius, Klaus Rabe, Werner Seeger and Tobias Welte – proposed to dedicate their Balzan Prize funds to the cross-disciplinary project *Effects of Short- and Long-Term Exposure to E-Cigarette Vapour*. The project is divided into three subprojects, listed below. A group of DZL early career scientists started Subprojects 1 and 2 in 2020; Subprojects 3 started in 2021.

1. Early responses to e-cigarettes and their components: airway structural changes, signaling pathways and survival in *Drosophila melanogaster* in the context of risk alleles (DZL site ARCN)
2. Effect of short- and long-term exposure to e-cigarette vapour on inflammation, endothelial barrier function and structural alterations in murine lungs (DZL sites BREATH and UGMLC)
3. Association of e-cigarette use with respiratory status in lung-healthy subjects and pulmonary patients (DZL sites CPC-M and TLRC)

In 2021, the COVID-19 pandemic still caused temporary closures and delayed progress, so adjustments to project management had to be made.

Scientific Report:

Subproject 1: Early responses to e-cigarettes and their components: airway structural changes, signaling pathways and survival in *Drosophila melanogaster* in the context of risk alleles (Dr. Hanna Angstmann, Prof. Dr. Susanne Krauss-Etschmann, Research Center Borstel, DZL site ARCN)

In the previous period, clear morphological and transcriptional changes in the fly airways of offspring

whose mothers had been exposed to vapoured nicotine (e-nicotine) had been observed. However, formal proof that vaping products indeed enter maternal airways – and not via the maternal cuticle or the eyes – was lacking. As vaping products are hard to visualize, a step backward was taken by using conventional tobacco smoke in larvae.

The fly´s anterior breathing openings, termed spiracles, are in the first thoracic segment, and the posterior ones are in the eighth abdominal segment (figure 1, bottom). Since the anterior spiracles are not functional until late larval stages, the question as to whether smoke passes beyond the posterior openings and pervades the entire tubular organ arose. To clarify this, *cyp18A1* expression was analysed in isolated and transversally bisected tracheae from male and female larvae. Cyp18a1 corresponds to the human homologue Cyp1a1, which metabolizes polycyclic aromatic hydrocarbons present in smoke. Both sexes responded to smoke with a several-fold increase in *cyp18a1* expression; however, tracheae from male larvae had a 20-25% lower *cyp18a1* transcriptional response than the female trachea (figure 1, top).

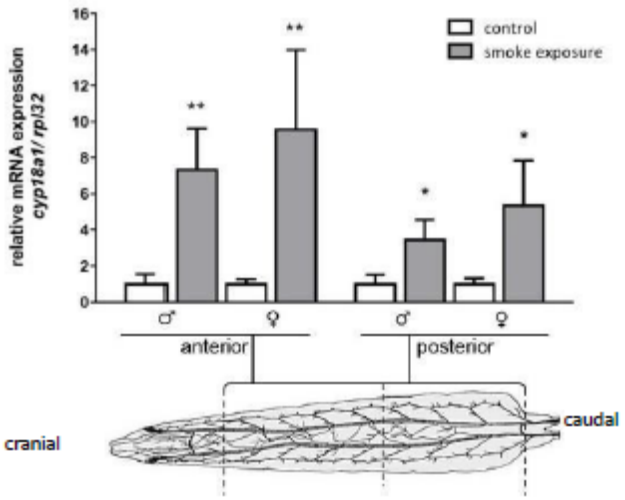

Figure 1: Cyp18a1 expression is markedly increased in the anterior parts of the airway tree compared to the posterior ones after smoke exposure.

To further corroborate these findings, RNA sequencing of isolated airways was performed, which demonstrated a clear difference between smoke exposed larvae and controls (figure 2), Moreover, a very clear difference between male and female larvae was seen.

Analyses of signalling pathways showed that affected genes were associated with oxidative stress response, fibroblast growth factor (FGF) signalling, innate immune response and G-protein coupled receptor (GPCR) signalling in both sexes. However, for some of these pathways, the expression profile was sex dependent. While a high number of genes involved in GPCR signalling and innate immune response was upregulated in females, they were mostly downregulated or proportionately up- and downregulated in males. Among the pathways altered exclusively in female larvae, cell adhesion, glutathione metabolic process, apoptosis, s and open tracheal system development had upregulated genes.

Thus, interesting sex-specific regulated pathways involved in respiratory system development and immune response were discovered within our study, highlighting the importance of studying the effect

of e-cigarettes separately in males and females as a next step.

Another manuscript has been submitted (Sirocko *et al.*).

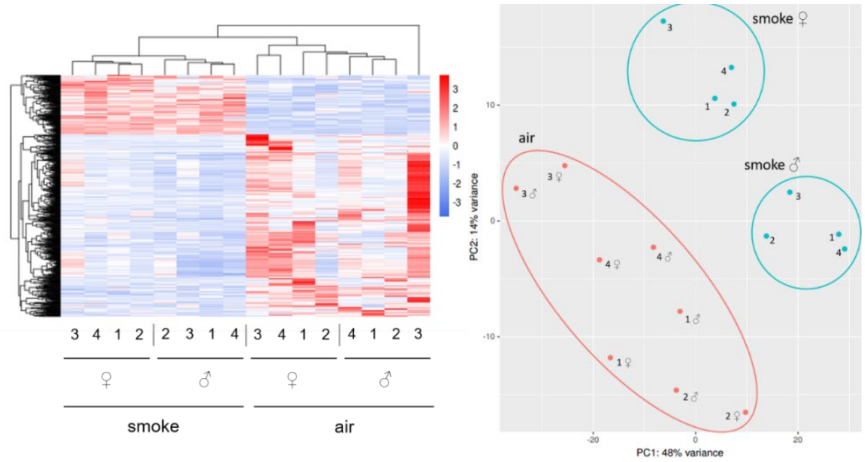

Figure 2: Transcriptomic analyses of dissected larval airways after cigarette smoke exposure (CSE). Airways of larvae were isolated within 1,5 h after CSE. A) Heatmap of all regulated genes in CS exposed female and male larval airways. B) Two-dimensional principal component plot showing the discrimination between tracheal gene expression profiles smoke exposed animals (blue) and controls (red). Each data point represents one sample containing 40-50 larval airways.

Subproject 2: **Effect of short- and long-term exposure to e-cigarette vapour on inflammation, endothelial barrier function and structural alterations in murine lungs – preliminary results** (Prof. Dr. Sascha David & Dr. Benjamin Seeliger, Hannover Medical School, DZL site BREATH; PD Dr. Natascha Sommer, Justus-Liebig University, Giessen, DZL site UGMLC)

BREATH subproject: Effect of exposure to nicotine containing e-cigarette vapour on endothelial barrier integrity in human-umbilical cord endothelial cells (HUVECs)

In the subproject at BREATH, the effect of e-cigarette vapour extract (ECVE) on endothelial barrier integrity in human umbilical cord endothelial cells (HUVECs) is studied. Previously, it was shown that incubation of HUVECs with ECVE with or without nicotine-containing medium lead to a dose-dependent increase in supernatant angiopoietin-2 (angpt-2) with nicotine-containing ECVE, and – to a lesser degree – without nicotine compared to control medium. An analysis was then carried out of the response to later stimulation with lipopolysaccharide (LPS) following pre-incubation on both the Angio- poietin/Tie2 system and continuous transendothelial resistance (TER) measurements using an electrical cell impedance sensing (ECIS) device.

Pre-incubation with ECVE medium increased the susceptibility of HUVECs to subsequent LPS stimulation by 20% with regards to ANGPT-2 production compared to control medium (figure 3A). The ANGPT2/TIE2 mRNA ratio increased significantly with nicotine exposure comparable to effects of LPS alone and was even higher with nicotine and LPS co-stimulation (figure 3B). On the functional level, pre-treatment with nicotine-containing ECVE resulted in an increased susceptibility to LPS-induced reduction in endothelial barrier integrity, as assessed by reduced transendothelial resistance (TER) in ECIS (figure 3C).

Preliminary *in vitro* data indicate that e-cigarette vapour can induce baseline endothelial activation and increase its susceptibility to subsequent LPS stimulation. Increased susceptibility to LPS-induced endothelial barrier breakdown in nicotine-containing ECVE-treated cells, suggests an increased vulnerability to sepsis-induced capillary leakage.

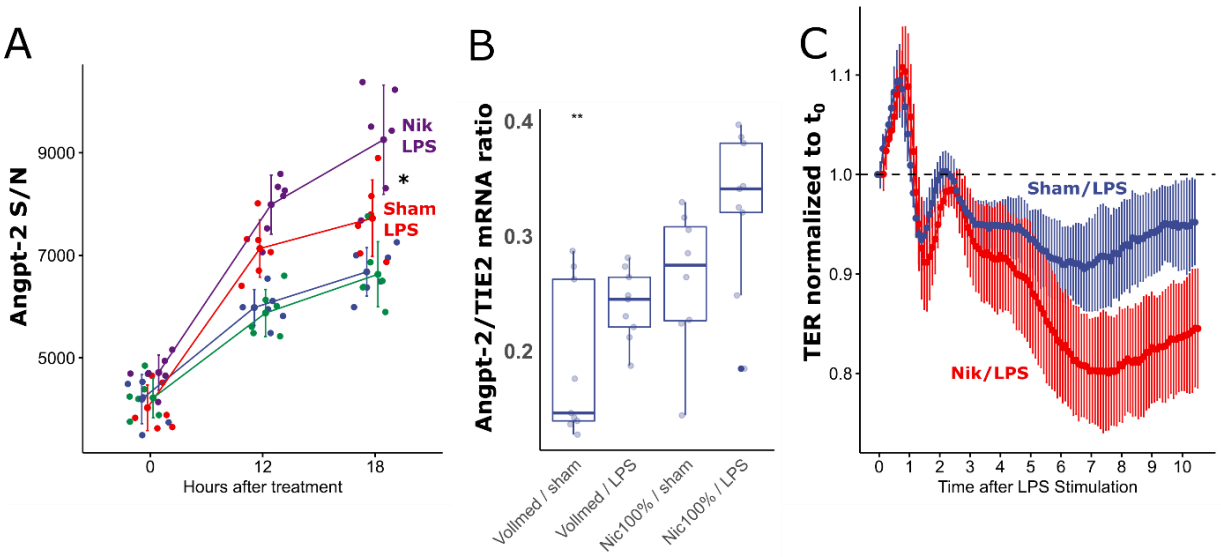

Figure 3: Following LPS stimulation, angiopoietin-2 (Angpt-2) production increased significantly in supernatant with nicotine-containing ECVE ("Nik") pre-incubation compared to LPS stimulation without ECVE (A). The ratio of resulting mRNA production of Angpt-2 and TIE2 increased with nicotine-EVCE ("Nic") similarly to LPS and was even higher with LPS stimulation after nicotine-ECVE pre-incubation (B). Continuous transendothelial resistance (TER) measurement showed a persistent decrease with nicotine-ECVE/LPS compared to LPS alone (C).

ECVE effects on transendothelial migration of monocytes through HUVECs and potential therapeutic options to antagonize these effects including selective TIE2-receptor activating monoclonal antibodies is now assessed.

UGMLC subproject: Effect of short- and long-term exposure to e-cigarette vapour on inflammation, endothelial barrier function and structural alterations in murine lungs

The UGMLC subproject investigated the short-term effects of different e-cigarette vapor (ECV) compounds on the endothelial barrier function of isolated mouse lungs. Previously, it was shown in isolated ventilated and perfused mouse lungs that direct inhalation of e-cigarette vapour induced endothelial dysfunction depending on the presence of nicotine or cherry flavour in the e-cigarette fluid. In contrast, the base component (propylene glycol, vegetable glycerin and water) alone did not significantly affect endothelial permeability. These results were reproduced in a second set of experiments during co-stimulation with two different inflammatory stimuli. Interestingly, an inflammatory co-stimulus did not aggravate e-cigarette smoke induced endothelial dysfunction (figure 4, shown for LPS).

Furthermore, the effects of eight months of exposure to e-cigarette vapour with or without nicotine in mice were investigated. Similarly to the experiments on isolated lungs, e-cigarette vapour containing nicotine showed a more pronounced effect on pulmonary alterations (induction of inflammation and parenchymal alterations) than e-cigarette vapour without nicotine. A manuscript is in preparation.

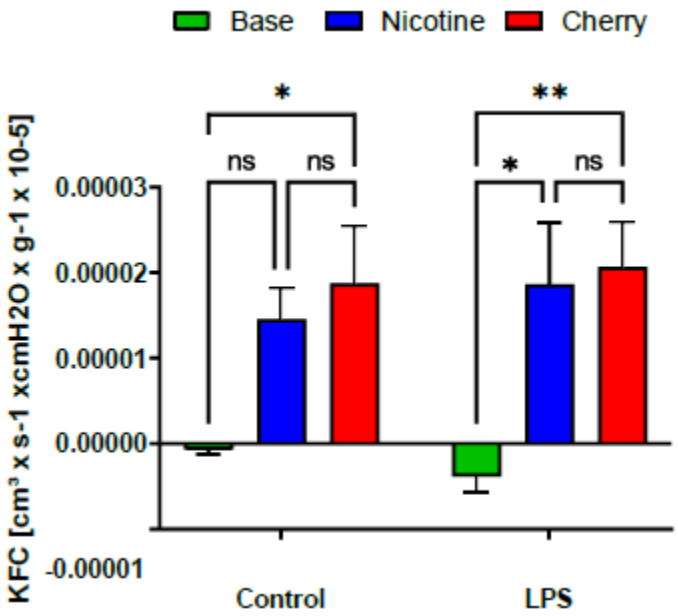

Figure 4: Capillary filtration coefficient (KFC) determined in isolated ventilated mouse lungs during direct inhalation with e-cigarette vapour via the trachea. Kfc was determined in absence (Control) and presence of lipopoly- saccharide (LPS).

Subproject 3: Association of e-cigarette use with respiratory status in lung-healthy subjects and pulmonary patients (Dr. Stefan Karrasch, LMU Munich, DZL site CPC-M, and Dr. Claudius Melzig, Heidelberg University Hospital, TLRC)

In this subproject, patterns of e-cigarette use and associations with conventional smoking as well as respiratory symptoms in subjects from the German National Cohort (GNC, NAKO Gesundheitsstudie) were examined. To further enable identification of potential imaging biomarkers based on magnetic resonance imaging (MRI), a cross-validation approach including imaging subgroups of both the population-based GNC (≈30,000 participants) and the COSYCONET cohort of COPD patients (≈500 patients) was chosen. COSYCONET, the German COPD and SYstemic consequences-COmorbidities NETwork, is an associated partner of DZL. Besides clinical examinations and pulmonary function tests, MRI examinations of the thorax were performed in these subgroups of participants in both studies.

Information on e-cigarette use was available in more than 64,000 adults aged 20 to 75 years, smoking or never smoking conventional cigarettes; about half of them were female. Overall, almost 10% of these participants, more men than women, reported to have ever used e-cigarettes. However, about 80% of these no longer used e-cigarettes at the time of examination. As expected, the percentage of ever-users was highest in the youngest age group and decreased with age. In preliminary analyses, the majority of examined e-cigarette users were dual users of conventional cigarettes and e-cigarettes, and a tendency towards a positive association regarding the intensity of both types of smoking was observed. Furthermore, e-cigarette users were more likely to report respiratory symptoms, especially coughing, even when taking possible concomitant effects of conventional smoking into account. These preliminary results indicate that e-cigarette use may have adverse effects on respiratory health in addition to the well-described impact of conventional smoking already affecting the typical e-cigarette users in young adulthood.

To derive lung-specific imaging parameters (lung volume and shape, vessel morphology, radiomics features) from underlying image data in GNC and COSYCONET, a voxel separation of the lungs from

surrounding tissues is required (image segmentation). Deep learning-based algorithms promise fast and fully automatic image segmentation. However, manual delineation of lung borders in a small number of participants is required as training data for the algorithm. A common approach for manual segmentation in both study cohorts was established and training datasets were created. Automatic lung segmentation was successfully performed and iteratively refined in the currently available datasets of ≈11,000 participants in the GNC and of ≈500 patients in the COSYCONET study. However, final validation of lung segmentations for further analysis requires visual evaluation of all segmentations in both cohorts (>30,000 cases). Therefore, visualisation methods for time-efficient identification of segmentation errors were developed during the past year and are currently being investigated for application in this project. As a next step, final evaluation of lung segmentations will be carried out and association of derived imaging parameters with smoking status and respiratory status will be analysed.

Outlook

The third subproject was started this year as intended. One initial paper had already been published in 2020. Publication of papers on the following subjects is also expected: a) transcriptomic analyses of e-nicotine exposed fruit flies; b) the influence of e-nicotine containing vapour on endothelial dysfunction in mice in the course of this year.