The impact of smoking-associated aldehyde exposure on the molecular regulation of mitochondrial function in epithelial cells of the airways and lungs

Impact paragraph

Worldwide tobacco epidemic – societal impact

Although it is known for years that tobacco smoking is harmful for health and a preventable cause of death, smoking is still a worldwide problem as 22.3% of the global population is using tobacco products. In addition, more than 8 million people die due to tobacco smoking globally each year, including 1.2 million deaths due to second-hand tobacco smoke (1). Also in the Netherlands, 1 out of 5 people is smoking and smoking is an important cause of illness and death as approximately >19.000 smokers die yearly (2, 3). Moreover, tobacco use has substantial societal and economic effects as 9.4% of the burden of disease is due to smoking in the Netherlands (2).

Last decades, several measures are taken by the World Health Organization (WHO) Framework Convention on Tobacco Control (FCTC) and the WHO Study Group on Tobacco Product Regulation (TobReg) to address the worldwide tobacco epidemic. Their focus is to prevent initiation of tobacco use, promote cessation of tobacco use and protect against exposure to second-hand tobacco smoke. Altogether, these measures aim to reduce tobacco-related morbidity and mortality. Moreover, WHO FCTC acknowledges that regulation of contents and composition of emissions of tobacco products (Articles 9 and 10) is required in order to reduce attractiveness, addictiveness as well as toxicity of tobacco products. Although quitting smoking is the preferred option to reduce the detrimental impact of tobacco on human health and the economy, less harmful tobacco products can be beneficial for the smokers' health given the addictiveness of tobacco consumption. Regulation of chemicals present in tobacco products is largely lacking, except for nicotine, carbon monoxide and tar. Moreover, it is not conclusively proven yet that lowering of specific components in tobacco will contribute to a reduced risk of mortality and morbidity of smokers. Based on toxicity data, one of thousands of toxicants suggested to be potential for mandated lowering by the WHO FCTC are the chemical group of aldehydes which, in the context of cigarette smoke (CS), mainly include acetaldehyde, acrolein and formaldehyde. (4, 5)

With regard to the morbidity and mortality numbers described above, it has to be mentioned that tobacco smoking is an important main risk factor for several respiratory diseases including chronic obstructive pulmonary disease (COPD) (6). COPD is a leading cause of death globally (7, 8). Mainly symptomatic treatments are available focusing on ameliorating lung function and reducing exacerbation (risk), however no cure exists for COPD patients (6, 9). COPD is a multifactorial disease in which, besides inflammation and oxidative stress, mitochondrial dysfunction is suggested to play a role (10, 11). Nowadays, mitochondrial dysfunction has been firmly implicated in the pathogenesis of COPD (12, 13), and CS-induced abnormalities in mitochondrial morphology and function have been described in literature (12-16). However, the underlying molecular mechanisms controlling mitochondrial content, metabolism and function (mitochondrial biogenesis, mitophagy, mitochondrial dynamics) in (epithelial)

cells of the lungs and airways are incompletely understood in COPD patients as well as upon exposure to CS and CS-associated aldehydes. Therefore, the aim of this thesis was to investigate the impact of several aldehydes (relevant for CS-exposure) on the molecular pathways regulating mitochondrial function in epithelial cells of the lungs and airways in the context of COPD.

Impact on fundamental and applied science

Our findings in **Chapter 2** provided novel insights in the extent of abnormalities in the molecular regulation (mitochondrial biogenesis *versus* mitophagy) of mitochondrial metabolism in COPD peripheral lung tissue. Directionality of the observed alterations in gene- and protein expression was dependent on disease status, showing more pronounced changes in very severe COPD patients. Moreover, as we observed discrepancies in the findings in different (cellular) compartments of the airways and lungs, this data emphasized the importance of using models reflecting different parts of the airways and lungs. Based on our results, the next step is to investigate if the observed alterations in mitochondrial turnover processes in lung tissue homogenates play a significant role in the pathogenesis of COPD, are responsible for alterations in mitochondrial function or may represent compensatory mechanisms. Our findings accompanied by additional data from future studies would contribute to filling this knowledge gap and discovering potential new therapy targets for COPD.

As in science there is an urgent request for transition from use of *in vivo* to *in vitro* models, we contributed to this aim by analyzing the relevance and applicability of various cellular and smoke exposure *in vitro* models in the context of our research question. The findings in **Chapter 3** highlighted the importance of making a thought-out choice for an *in vitro* exposure model, which is tailored to the specific research question. By comparing more advanced models using differentiated primary bronchial epithelial cell cultures exposed to whole CS to more simple conventional models using submerged primary bronchial epithelial cell cultures and CS extract, we aimed to encourage future inhalation toxicology researchers to carefully consider if and when it is needed to use more complex models over the more simple *in vitro* models. This study hopefully creates more awareness for the pitfall to assume that more complex models are 'better', while in particular cases the simple models are sufficient to answer the research question and thereby safe time and money.

Taken into account the findings of **Chapters 2 and 3**, our studies support the value of comparison of various experimental models to consider applicability for respiratory (toxicology) studies. In the future, even more complex models mimicking the human lung environment (e.g., co-cultures, organs-on-chip) should be included in this research field.

Furthermore, in this thesis, we studied the regulation of mitochondrial metabolism in response to noxious particles, i.e., CS and aldehydes, in several *in vivo* and *in vitro* models. We observed differences in degree of effect of CS, mix of aldehydes or acrolein exposure *in vivo* or *in vitro*, following an acute or sub-acute exposure regime (**Chapters 4, 5, 6)**. Heterogeneity observed in the results of these studies could be explained by the variety in models used (*in vivo* rat (lung) *versus in vitro* human primary bronchial epithelial cells), route (nose-only, whole-body) and duration of exposure (acute *versus* sub-acute), mixture (CS or three aldehydes) *versus* the individual aldehyde acrolein, and dosimetry. These studies elaborate the knowledge about fundamental pathways involved in the regulation of molecules associated with mitochondrial biogenesis, mitophagy and mitochondrial dynamics in response to CS or aldehydes, which is a suggested mechanism to predispose to COPD. Future studies could focus on the (potential additive/synergistic) impact of (other) compounds (individually or in a mixture) emitted by CS on the function of mitochondria to contribute to and/or strengthen the evidence reported in our studies. It is important to take into account the translational impact of our findings to the human situation, but also to consider what is the most informative and applicable model matching the research question. This data again underscores the importance of a considered choice for the use of a specific research model. The innovative model developed in **Chapter 6** contributes to the transition to *in vitro* research (replace). This model (puff-like exposure, differentiated cultures) could be of value for future research in the field of inhalation toxicology of noxious particles and unravelling molecular mechanisms underlying respiratory diseases. Moreover, we also used lung material from animal studies which were designed and conducted by collaborating research groups (reduce; **Chapters 4 and 5**) and we build on their already published findings (17, 18). By reusing these samples from previous executed animal studies, the needless repeating of animal studies and corresponding unnecessary use of animals is prevented. In conclusion, lessons learned from our studies could be used for design of future studies in this research field, taking into account the challenges faced regarding dosimetry (e.g., dose, peak-exposure mimicking puff topography), mixture toxicology, exposure duration and route, and model used.

Lasty, the knowledge from our studies combined with additional insights about the causal and mechanistic impact of several aldehydes (relevant for CS-exposure) on the molecular pathways controlling mitochondrial content, mitochondrial function and metabolism in epithelial cells of the lungs and airways in the context of COPD could contribute to detect, investigate and develop potential therapeutic applications targeting aldehydes-induced mitochondrial dysfunction in COPD therapy in the future.

Impact on regulation

Despite the initiation by the WHO FCTC to approach the tobacco epidemic by aiming to reduce harmful components present in CS, minimal progress has been made last years with respect to regulation of specific chemicals. This research project aimed to provide additional scientific evidence to fill the knowledge gaps regarding the impact of aldehydes emitted by CS on epithelial cells of the airways, in particular related to the molecular regulation of mitochondrial content/function. The evidence described in this thesis aims to provide, together with other (previous) research, a scientific basis to potential regulate aldehydes content/composition of emissions in CS in the future. Nevertheless, as described in **Chapters 4, 5, 6** our findings regarding the impact of a mixture of aldehydes *versus* individual aldehydes were inconclusive making it hard to draw any conclusions and recommendations for future regulation only based on our data. Therefore, policy makers should critically review and take into account all available scientific literature and acknowledge the current knowledge gaps in their consideration for the mandated lowering/regulation of aldehydes ((added) sugars) in CS and limit emission of CS-associated aldehydes in tobacco products. Our research creates more awareness of the need and potential for regulation of the contents and composition of emissions of tobacco products, in particular sugars and additives in tobacco (smoke). Decreasing sugar yield, selection of specific tobacco types and design of a cigarette (e.g., cigarette filter ventilation) are ways to reduce aldehyde content in cigarettes (19-21). In addition, the amount of aldehydes exposure of a smoker depends on smoking behavior/topography (21). In follow-up of our research, additional studies are necessary to prove if a reduction of specific aldehydes in CS is required and to what extent this should be in order to induce a beneficial health effect for smokers in relation to mitochondrial (dys)function and COPD development. In addition, based on our findings, it is interesting for other researchers to investigate the impact of exposure to aldehydes or other CS-components alone or possible (additive/synergistic) interaction with other compounds in the mix of thousands of chemicals present in CS.

Target groups

The findings from this thesis are relevant for several target groups. First and foremost, the research described in this thesis is relevant for other researchers in the field of respiratory diseases and inhalation toxicology. We aimed to contribute to the knowledge about the underlying molecular mechanisms involved in the regulation of mitochondrial content, function and metabolism in (epithelial) cells of the airways and lungs in response to smoking-associated aldehydes exposure in the context of COPD. By investigating this, we hope to extend knowledge in this research field, set a basis for further research and might reveal therapeutic targets for this disease. With respect to inhalation toxicology, we also aimed to provide insight in differences and similarities

between the various *in vitro* models and *in vivo* models, emphasizing the importance of tailoring the model to the research question. In addition, we deployed a sophisticated (puff-like) exposure model for differentiated primary bronchial epithelial cells to a mixture of aldehydes and CS, which could be used in future research in the field of inhalation toxicology but is also applicable to research disciplines outside this area. Secondly, as described in the paragraphs above, our study is relevant for policy makers. Although our findings did not provide conclusive evidence for regulation of aldehydes in CS, it is relevant for policy makers to include our findings in the review of available scientific literature to consider the potential (health) impact of the regulation/lowering of aldehydes ((added) sugars) to the emission of CS-associated aldehydes in tobacco products and acknowledge the current knowledge gaps.

Conclusion

In conclusion, the research described in this thesis provides a comprehensive overview of the impact of CS and aldehydes on the regulation of processes involved in mitochondrial content, function and metabolism in the context of COPD using various *in vitro* and *in vivo* exposure models of the airways and lungs. By comparing various experimental models, our study highlights the importance of making considered choices for experimental model and exposure type/regime tailoring the research question. Moreover, our data contribute to the knowledge about aldehydes-induced mitochondrial toxicity in (epithelial) cells of the lungs and airways. These findings may be of value for both researchers in the field of respiratory diseases and inhalation toxicology. Furthermore, our study provided additional data which could be taken into account by policy makers to consider potential (health) impact by future regulation (lowering) of aldehydes levels in CS. Altogether, this thesis contributes to reducing the detrimental impact of smoking on society, economy and health status, in particular with regard to COPD.

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