Exploring Gene Expression Variability: Insights from Rat Lungs and Human Precision-Cut Lung Slices analyzed with qPCR

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1. Abstract

This diploma thesis aims to advance in vitro-in vivo extrapolation (IVIVE) efforts to replace traditional rat models with non-animal-based models, aligning with the 3Rs principles. Gene expression related to inflammation and fibrosis was compared using rat and human Precision Cut Lung Slices (PCLS) models. Both models were exposed to DQ_{12}, a nanoparticle known to induce lung inflammation and fibrosis. Gene expression was measured via qPCR. Results from rat samples showed only in CXCL5-6 a significant increase in expression at 24h compared to the control group. Human PCLS sample sizes were insufficient for statistical analysis. Further tests are needed to align the PCLS model with the rat model.

2. Introduction

Nanoparticles can cause oxidative stress, leading to inflammation and fibrosis in the lungs[1]. They occur naturally and are produced in industries like manufacturing, where exposure poses health risks, necessitating thorough inhalation toxicology testing. Current models primarily rely on animal testing, particularly in rats, but this has limitations due to anatomical and physiological differences between species.[2] Additionally, there are growing ethical concerns about animal testing. The scientific community has adopted the 3Rs principle: Replacement, Reduction, and Refinement for animal use in research.[3]

This thesis explores how the in vitro model, human PCLS, can be aligned with the in vivo, rat based, model. Aiming to improve testing approaches and reduce the need for animal experiments.

3. Aims and leading questions

The aim of this study is to compare gene expression between rat lung samples and human PCLS samples using qPCR. New primers were designed for both rat and human samples. The qPCR results were analyzed and visualized using bar graphs.

4. Methods and Material

<u>Human PCLS:</u>
PCLS allow for the study of lung tissue in vitro while preserving its architecture and cellular complexity. Human lung samples obtained from surgical cancer procedures are infused with agarose, solidified, and cut into thin sections using a vibratome. The cells in these samples remain viable in cell culture, where they are exposed to a dose of 1 µg/cm² and 10 µg/cm² DQ_{12}. Samples were collected at the time points of 24h, 7d, and 14d after exposure.

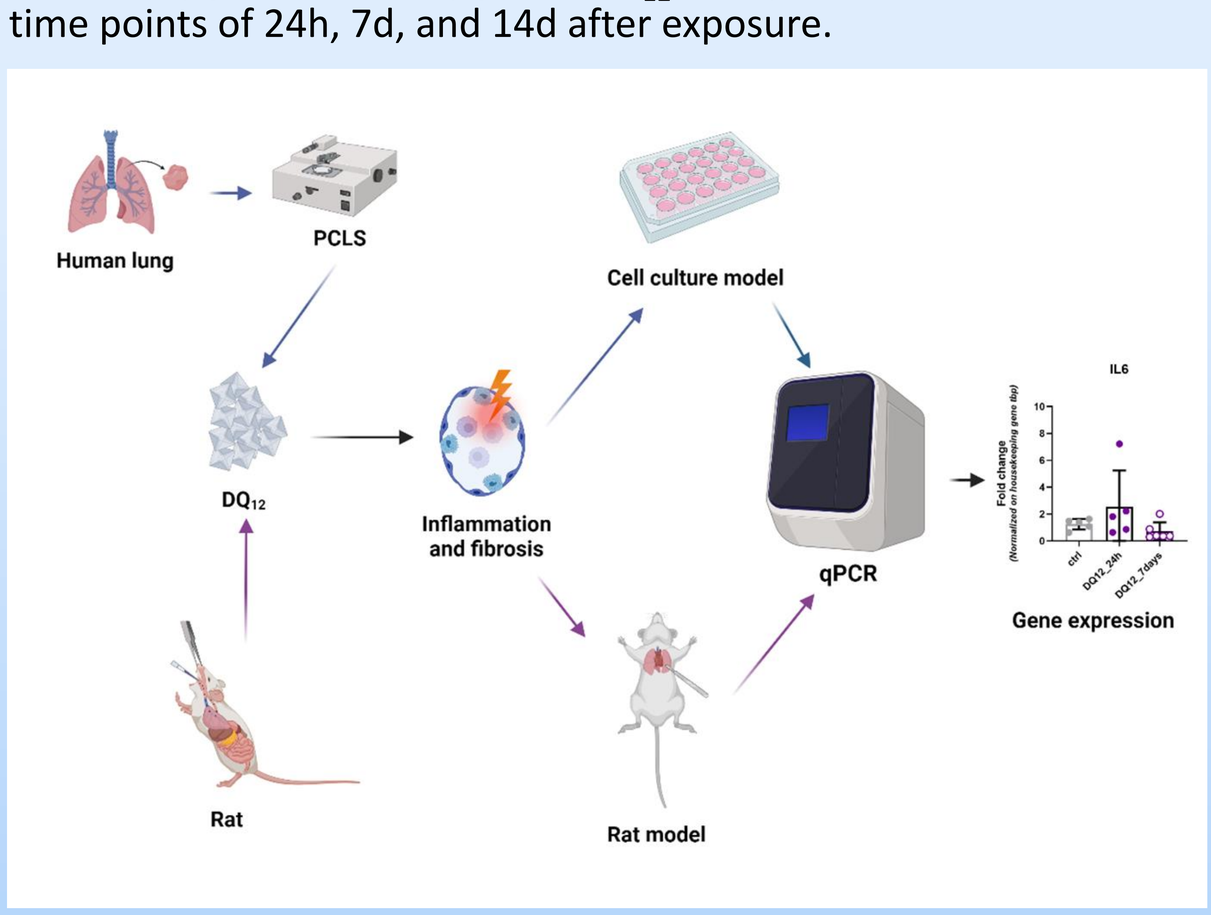


Fig. 1: Experimental Procedure (Ringger, 2024)

<u>Rats:</u>
Rats were exposed through oropharyngeal aspiration to a concentration of 8mg DQ_{12}/kg. Following the exposure period, the rats were kept under normal conditions. Samples were collected at 24h and 7d, after euthanasia.

The collected samples were analyzed using qPCR, allowing for the quantification of RNA and thus gene expression, as shown in Figure 1. New primers were designed and tested for this process.

Gene analyzed were chosen for their involvement in the inflammatory response and in the case for TGFβ for the involvement in the fibrotic process[4]. Genes analyzed:

Rat model: TNFα, TGFβ, IL6, IL12α, IL18, CXCL1, CXCL5-6 and tbp

In PCLS model: TNFα, TGFβ, IL6, IL12α, IL18, IL8, IL1β, GAPDH and YWHAZ

Tbp and GAPDH/YWHAZ were used as housekeeping genes. IL8 is not present in rat DNA, instead CXCL1 and CXCL5-6 are expressed as close analog to the human IL8.

5. Results

Results were analyzed regarding normal distribution. As statistically significant a p value of <0.05 was utilized. In rats, no significant results were found, except for CXCL 5-6. For PCLS the samples size was too small for statistical analysis.

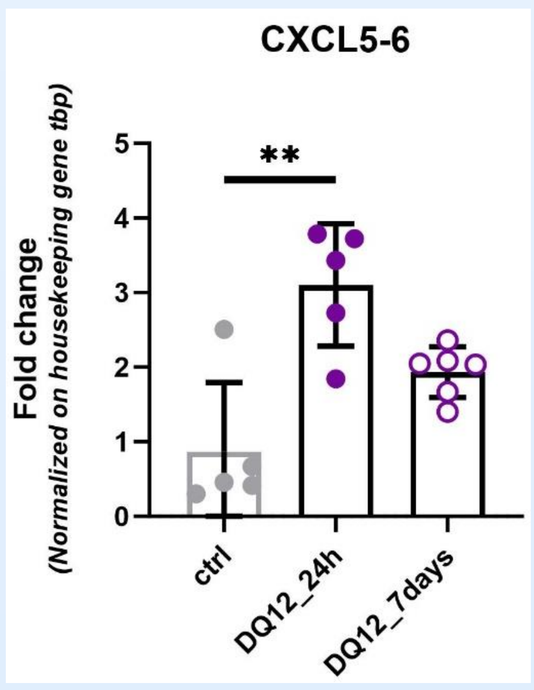


Fig. 2: *Bar chart CXCL5-6 in rat* (Ringger, 2024)

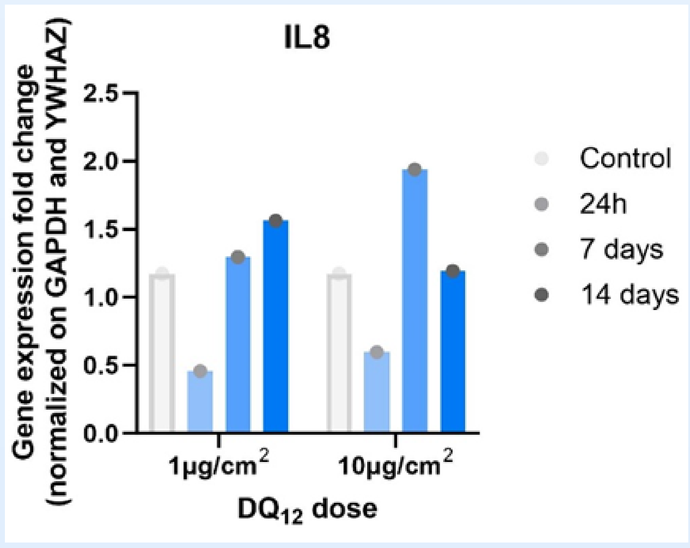


Fig. 3: *Bar chart IL8 in human* (Ringger, 2024)

6. Discussion and conclusion

In rats, CXCL5-6 is the only gene that shows significant changes from the control group to the 24h time point. For human PCLS, it was not possible to determine if the results were significant due to the low sample size. The gathered results suggest that there is no increase in gene expression of IL8 at the 24h time point. Which does not align with the results from the rat model. For confirmation, further testing is needed.

rimer testing showed promising results; however, due to ongoing challenges with gel electrophoresis, addressing these problems is still ongoing.

These results represent a small step toward aligning in vivo and in vitro models, contributing to the long-term goal of replacing, reducing, and refining animal experimentation in inhalation toxicology.

References:
[1] Kreyling et al., 2010
[2] Movia et al., 2020
[3] Russell & Burch, 1992
[4] Loncarevic et al., 2024, submitted
[5] Seyran Mutlu, Personal communication, January 2023

Figure:
Fig. 1 Ringger, L. (2024). [In BioRender.com] medi.
Fig. 2 Ringger, L. (2024). [In GraphPad PRISM] medi.
Fig. 3 Ringger, L. (2024). [In GraphPad PRISM] medi.