SUMMARY

Introduction

Bronchopulmonary dysplasia (BPD) is the most common chronic lung disease in extremely premature infants, with significant short- and long-term consequences. BPD pathogenesis is multifactorial, comprising pre- and postnatal infections, oxygen toxicity, and mechanical ventilation. The so-called new BPD of the surfactant era is characterized by abnormal lung development and comprises a severe depletion of alveoli, disturbed vascularization, and pathological airway changes, which lead to diminished gas exchange in neonatal patients. Nowadays, airway remodelling is mainly investigated by utilizing different staining protocols applied to lung tissue sections. However, the data do not provide information on a molecular level. Infrared microspectroscopy has high molecular sensitivity and, with a combination of multivariate data analysis, provides quantitative and qualitative information on biological molecules, providing a "biochemical fingerprint" of the analyzed structure. Therefore, in the present study, the possibilities of this method have been used to analyze lung tissue of the BPD animal model.

Aim of the study

The study aimed to analyze lung tissues of the BPD animal model and compare the obtained results with healthy control.

Material and methods

All experiments were performed in the Hudson Institute of Medical Research and Australian Synchrotron in Australia. Murine model of BPD was induced by perinatal inflammation and placement of the newborn pups either in the hyperoxia (65% of oxygen, test group) or normoxia (21% of oxygen, control group). On day 28, pups were humanely killed, and lungs were dissected, fixated, and paraffin-embedded. For microscopic and spectroscopic examinations, different thicknesses of lung tissue sections from ten mice in the control group and eleven mice in the test group were used. Tissue sections for morphometric examination were stained with hematoxylin and eosin. The remaining sections were examined with FPA-FTIR for airways and S-FTIR for alveoli. Spectra has been collected in transmission mode in the wavenumber range 4000-900 cm-1 for airways and 3800-700 cm-1 for alveoli, with 4cm-1 spectral resolution. Each sample was scanned at least three times in different regions related to airways or alveoli walls, previously selected on compatible, stained sections. After the initial

processing of chemical maps, the spectral description was performed. The absorbance coefficients were calculated in the next stage, and collected spectra were analyzed with PCA.

Results

All performed analyses showed clear differentiation between the lung tissue of diseased and healthy individuals. Morphometric analysis showed a 60% reduction in alveolar number, an 80% decrease in the gas exchange area, and a twofold increase in alveolar size in the test group. Molecular changes in airway walls are mainly observed by the increase in the amount of α-helical conformations of amide I and the increase in collagen and tyrosine. The molecular changes in alveoli comprise quantitative changes in amide I and II conformations, collagen, tyrosine, DNA, and RNA, as well as conformational changes of these compounds. The collected data confirm the "new" BPD pathology, where minor changes in airways and more significant changes in alveoli are observed.

Conclusions

1. FTIR microspectroscopy is a sensitive tool for the characterization of lung changes associated with bronchopulmonary dysplasia in the animal population (mice).
2. There is a high agreement between the obtained results and results based on other methods used for examining pathological changes in bronchopulmonary dysplasia.
3. Since the spectral and chemometric analyses of airways showed considerable agreement between the data sets from different time intervals, it is possible to construct a classification model based on the obtained results. However, it is beyond the scope of this study.
4. There is a need to conduct further studies using an additional complementary method such as Raman spectroscopy, visible light, and ultraviolet spectroscopy, or nuclear magnetic resonance spectroscopy.
5. There is a need to research a larger population, which will increase the statistical reliability of the results.

Key words: bronchopulmonary dysplasia, FTIR spectroscopy, airways, alveoli