SUMMARY

Introduction

It is estimated that air pollution is among the top 10 risk factors for death in the world. Cracow is one of the most polluted cities in Europe in terms of particulate matter 2.5 ($PM_{2.5}$) concentrations in the air. Atherosclerosis is implicated in the pathogenesis of numerous cardiovascular diseases and remains among the leading causes of morbidity and mortality worldwide. It is a multifactorial progressive disease characterized by inflammation of the vessel wall. Coronary artery disease involves the formation of atherosclerotic plaques in epicardial coronary arteries. There is growing evidence that long-term exposure to air pollutants (primarily $PM_{2.5}$) contributes to the pathogenesis of atherosclerosis and its complications, including coronary artery disease. Although the postulated pathomechanisms of air pollution side effects include inflammatory response, oxidative stress, endothelial dysfunction, and coagulation disorders, the exact mechanisms leading to the development of atherosclerosis remain unknown. Monocytes constitute a heterogeneous population of cells with different functions, phenotypes, and locations. They have been shown to be involved in the development of atherosclerosis, inflammation, and angiogenesis. MicroRNAs (miRNAs) are small endogenous non-coding single-stranded RNA molecules (typically consisting of 21 to 23 nucleotides) that play a significant role in regulating gene expression at the posttranscriptional level. Abnormal miRNA expression has been associated with numerous cellular processes and metabolic pathways implicated in the pathogenesis of various diseases, including those affecting the cardiovascular system. Current data suggest that miRNA signaling may be actively involved in the cellular response to different chemicals, including $PM_{2.5}$.

Aims

This study was designed to assess the impact of natural long-term seasonal (winter – summer) exposure to air pollution (especially $PM_{2.5}$) among the permanent residents of Cracow. Specifically, the aim of the study was to investigate whether the assumed higher concentrations of various air pollutants in winter lead to significant changes in gene expression (miRNA) and concentrations of proinflammatory cytokines. The clinical part of the study was designed to assess if miRNA expression and proinflammatory cytokine levels differ significantly between individuals with chronic coronary syndrome (CCS) and those without CCS. As part of the in vitro study, the following parameters were assessed: expression of selected miRNAs,

concentrations of selected proinflammatory cytokines, as well as activity and morphology of human peripheral blood monocytes exposed to $PM_{2.5}$ samples of known chemical composition and origin, collected in Cracow during winter and summer. The aim of the experiment – in combination with clinical results – is to explore the potential role of monocytes in the development of endothelial and atherosclerotic plaque inflammation following exposure to $PM_{2.5}$.

Materials and methods

The clinical part of the project was conducted as a prospective cohort study. It included 140 permanent residents of Cracow, recruited between 2017 and 2019 from among patients hospitalized at the Department of Coronary Disease and Heart Failure of Jagiellonian University Medical College. Study participants were divided into two groups of equal size. The research group included patients with CCS (n = 70), and the control group – patients without CCS (n = 70). Peripheral venous blood was collected twice from all participants (in the winter and summer seasons). The samples were secured for the subsequent evaluation of the expression of selected miRNAs and concentrations of specific proinflammatory cytokines. The effect of $PM_{2.5}$ on the activity of human monocytes was determined in cells derived from peripheral venous blood obtained from 3 healthy volunteers. $PM_{2.5}$ samples were collected seasonally (winter and summer) on the roof of the Faculty of Physics and Applied Computer Science's building at AGH University of Science and Technology in the Cracow district of Krowodrza. For an in vitro experiment (evaluation of the expression levels of selected miRNAs and concentrations of specific proinflammatory cytokines in the suspension culture of human monocytes incubated with $PM_{2.5}$ samples), a dry extract was prepared from selected samples separately for the winter and summer seasons. The remaining samples was used for a comprehensive and multicomponent analysis of $PM_{2.5}$ composition and concentration. The phagocytic activity of monocytes against $PM_{2.5}$ particles was assessed by using electron microscopy to examine the morphological changes of cells subjected to acute in vitro exposure to $PM_{2.5}$ samples.

Results

The study confirmed significant differences in the expression of selected miRNAs after stimulation of human monocytes with $PM_{2.5}$ samples collected in Cracow during winter and summer, when compared with unstimulated cells. Significant overexpression of miRNAs (miR-101-3p, miR-34c-5p, miR-223-5p, and miR-25-3p) was also shown in serum samples

obtained from participants during the winter when compared with the summer season. The assumed reproducibility of some miRNA patterns associated with inflammation and atherosclerosis (miR-101-3p, miR-34c-5p, miR-223-5p, miR-25-3p, and miR-382-5p) was confirmed both in the in vitro and clinical studies. This indicates that monocytes participate in the molecular pathways of atherogenesis after exposure to $PM_{2.5}$. Electron microscopy revealed increased phagocytic activity of monocytes exposed to $PM_{2.5}$ samples collected during the winter, as compared with unstimulated cells (without noticeable changes in monocyte morphology). $PM_{2.5}$ concentrations were higher in the winter than in the summer season. Similar findings were observed for to other air pollution components including elements (Cl, Mn, Fe, Ni, Cu, Zn, Br, Rb, Sr, and Pb), inorganic ions (SO_4^{2-}, NO_2^-, NO_3^-, NH_4^+, PO_4^{3-}, Cl^-, Li^+, K^+, Na^+, Ca^{2+} and Mg^{2+}), polycyclic aromatic hydrocarbons (mainly benzo[a]pyrene, benzo[a]anthracene, benzo[b]- and benzo[k]fluoranthene, indeno[1,2,3-cd]pyrene, pyrene and dibezno[a,h]anthracene), as well as elemental and organic carbon.

Conclusions

The study confirmed a negative association between air pollution ($PM_{2.5}$) and human health. The exposure of human peripheral blood monocytes to $PM_{2.5}$ was shown to cause significant changes in the expression of various miRNAs that regulate numerous cellular functions and possibly contribute to atherosclerosis and other disorders related to inflammation, oxidative stress, as well as cell proliferation and differentiation. The in vitro changes in monocyte expression of selected miRNAs as well as the seasonal changes in the miRNA profile in human serum suggest that monocytes are involved in the development of atherosclerosis following exposure to $PM_{2.5}$. Some of these miRNAs may represent new therapeutic targets for vascular proliferative diseases and serve as potential markers of exposure to air pollutants. The study also showed a worse air quality in Cracow during the heating season.