SUMMARY

Tobacco smoking is a major risk factor for development of chronic inflammatory diseases and cancers of the respiratory tract. The enzyme 12/15-lipoxygenase (12/15-LOX) plays an important role in the regulation of inflammatory response triggered by injurious agents by influencing the metabolism of arachidonic acid. The purpose of this study was to assess the role of 15-LOX in the expression of genes encoding proteins involved in the process of tissue remodelling and angiogenesis in the lungs exposed to tobacco smoke. The study was conducted on an animal model using mice deficient in the gene responsible for encoding 12/15-lipoxygenase (12/15-ALOX), which is the equivalent of 15LOX in humans, i.e. the so-called knockout mice (*12/15Alox*KO). There were a total of 120 mice, out of which 60 were deficient in the 12/15- lipoxygenase gene and the remaining 60 were wild-type mice without any genetic modifications. The animals were exposed to tobacco smoke for a period from 6 hours to 4 months. At specific time points (after 6 and 24 hours, 10 days, 4 weeks, 4 months) the mice were killed and samples of lung tissue were collected for tests. Histopathological examination looked at changes in lung structure (aggravation of emphysema) and the size of macrophage infiltration within lung tissue. The expression of selected genes was assessed based on the RT-PCR technique using TaqMan® Array Micro Fluidic Cards that allow for simultaneous testing of multiple genes. As angiogenesis plays a central role in chronic inflammation as well as regeneration and remodelling of tissues, the study used cards designed for testing 46 genes involved in the process of angiogenesis. It was demonstrated that the exposure to tobacco smoke caused emphysema in both wild-type and knockout mice. The changes were correlated with the time of exposure to tobacco smoke. Emphysema was more severe in 12/15ALOXKO mice compared to the control group of mice. There was also a statistically significant increase in the number of macrophages in the lung tissue of mice deficient in the 12/15ALOX gene in comparison to the wild-type mice. Analysis of gene expression it revealed that after exposure to tobacco smoke there were significant changes in the expression of 27 out of 46 analyzed genes, when compared to mice without tobacco smoke. The greatest dynamics of change in gene expression were observed during the initial 24 hours of exposure to tobacco smoke. Only 10 out

of the 27 genes exhibited changes in expression in both groups of mice and only 3 out of those followed a similar expression “pattern” in both groups. As for the other genes, there were significant differences in expression between the analysed groups. The largest differences in gene expression profile applied to the genes encoding growth factors and chemokines and cytokines. 12/15Alox silencing was associated with a significantly weaker induction of expression of genes encoding growth factors, which could be responsible for insufficient regenerative response of the respiratory system thus contributing to the aggravation of emphysema in that group of mice. On the other hand, in 12/15ALOXKO mice there was a significant increase in the expression of genes encoding chemokines, which could be related to the presence of larger inflammatory infiltrates consisting of macrophages in that group of mice after exposure to tobacco smoke.

The results of the study suggest that 12/15-lipoxygenase protects the respiratory tract from damage caused by chronic exposure to tobacco smoke. Modifying the activity of 12/15-lipoxygenase could therefore become a desirable goal of treatments aimed at preventing and/or stopping the progression of tobacco-related chronic respiratory diseases.