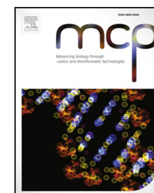
ARTICLE IN PRESS

Molecular and Cellular Probes xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Molecular and Cellular Probes

journal homepage: www.elsevier.com/locate/ymcpr



Changes in gene expression in lungs of mice exposed to traffic-related air pollution

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ARTICLE INFO

Keywords:
Traffic-related pollutants
Gene expression
PCR array analysis

ABSTRACT

Long-term exposure to traffic-related pollutants can lead to a variety of respiratory diseases, including inflammation, asthma, and lung cancer; however, the underlying biological mechanisms are not fully understood. We focused on the effects of exposure to different air pollutants on the expression of genes associated with inflammatory immune responses, allergic reactions and asthma, and lung cancer. In order to understand the cellular responses induced by exposure to different traffic-related pollutants, we performed PCR array to evaluate the mRNA expression of genes associated with inflammatory immune responses, allergic reactions and asthma, and lung cancer in the lungs of mice exposed to three different environments, including the laboratory (clean air), and polluted parking garages in Foshan and Guangzhou for four weeks. Cytokines (IFN-γ, IL-4, and IL-17A) were analyzed by Flow cytometry; the morphological structures were detected by Haematoxylin and eosin (H&E) staining. Our results revealed that the main pollutant in Guangzhou was PM2.5, the main pollutants in Foshan were gaseous pollutants including CO, NO_x and SO_2. IFN-γ was significantly lower, and IL-4, and IL-17A were significantly higher in mice in the Guangzhou and Foshan groups compared with laboratory group. The morphological structures were damaged in Guangzhou and Foshan groups. In addition, we found that exposure to traffic-related pollutants triggered the expression of inflammatory genes (Cxcl11 and Tnfs4), allergy and asthma genes (Clca3 and Prg2), and lung cancer genes (Agr2, Col11a1, and Sostdc1). As such, our results demonstrate that persistent exposure to traffic-related pollutants may elevate the incidence of immune disorders and asthma, and may be as a risk factor for lung cancer.

1. Introduction

In recent years, studies have shown that exposure to air pollution is associated with numerous diseases, especially cardiopulmonary diseases and lung cancer. For example, Gehring et al. [1] and Schultz et al. [2] have suggested that exposure to air pollution increased the risk of respiratory and allergic disorders. Research conducted by Vermeulen [3] has also demonstrated that exposure to air pollution increased the morbidity and mortality of lung cancer. Currently, antioxidant and inflammatory responses are thought to be the underlying biological mechanisms for the increased risk of respiratory and allergic disorders caused by exposure to air pollution [1,2]. Previous studies have confirmed that long-term exposure to particulate matter (PM) with a diameter of less than 2.5 μm (PM2.5) can accelerate the progress of many diseases, including asthma and lung cancer [3–5]. Furthermore, inhaled particulate matter and gaseous pollutants, such as NO_2, may play important roles in the developmental processes of diseases through stimulating the release of pro-inflammatory mediators [6,7].

Our previous study in cooperation with Sun Yat-sen University evaluated the effects of automobile fumes in underground parking lots on the immune systems of animals. The results indicated that long term exposure to a high concentration of pollutants promoted alveolar epithelium swelling and particle formation. Moreover, multiple inflammatory factors were increased in peripheral blood. We concluded that the air pollutants triggered immune disorders. However, the previous study did not further explore the expression changes of associated genes, and the parking space environment does not accurately represent the road traffic environment.

As such, this study expands on this previous research. In the current study, mice were divided into three groups, and each group was exposed to different kinds of air pollution in the laboratory, Guangzhou, and Foshan. Lung tissues and serum from mice in each group were

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https://doi.org/10.1016/j.mcp.2018.03.005
Received 3 February 2018; Received in revised form 16 March 2018; Accepted 31 March 2018
0890-8508/ © 2018 Published by Elsevier Ltd.

Please cite this article as: Yang, J., Molecular and Cellular Probes (2018), https://doi.org/10.1016/j.mcp.2018.03.005

collected. Cytokines (IFN-γ, IL-4, and IL-17A) were measured by Flow cytometry; the morphological structures were detected by Haematoxylin and eosin (H&E) staining. The expressions of genes associated with inflammatory immune responses, allergic reactions and asthma, and lung cancer were detected by PCR array and verified by RT-PCR. Our results demonstrated that the degree of inflammation was lower, and the morphological structure was more complete in laboratory group; persistent exposure to traffic-related pollutants may elevate the incidence of immune disorders, asthma, and lung cancer. Furthermore, the Clca3 gene and the Col11a1 gene were demonstrated to be related to the pathogenesis of diseases induced by exposure to atmospheric pollutants.

2. Materials and methods

2.1. Animals

Adult male BALB/c mice (8 weeks of age) were obtained from the animal experimental center in the North Campus of Sun Yat-sen University. Experiments were approved by the animal ethics committee. The mice were divided into three treatment groups (laboratory, Foshan, Guangzhou). Mice in the three groups were housed in the laboratory in the North Campus of Sun Yat-sen University (a clean air environment), site A (an underground parking lot in Guangzhou, Haizhu district), and site B (an underground parking lot in Foshan, Sanshui district) respectively. There were 15 mice in each group. All mice were maintained in a normal light-dark cycle, with available food and water for four weeks. Animals were euthanized by cervical dislocation, and the lung tissue was collected and stored at liquid nitrogen for gene expression analyses.

2.2. RNA purification

The total RNA of lung tissues was isolated using TRIzol® reagent (Gibco BRL) according to the manufacturer's specifications. First, the lung tissues were pulverized and homogenized in 1 ml of TRIzol. Then, the RNA was precipitated using isopropanol after chloroform addition and centrifugation. The RNA concentration was determined with a NanoDrop® ND-1000, and the integrity and purity of RNA were assessed using an Agilent 2100 Bioanalyzer.

2.3. Reverse-transcription real-time PCR and array analysis

The reactions of RT-PCR were carried out using the 2X SuperArray PCR master mix, and the PCR arrays were amplified with the ABI QuantStudio 12K Flex system. PCR arrays (96 wells) were designed for the evaluation of mice genes associated with inflammatory immune responses, allergic reactions and asthma, and lung cancer. The PCR arrays were conducted using the following kits from SABiosciences: mouse inflammatory cytokines and receptors, PAMM-011Z, mouse allergy and asthma, PAMM-067Z, and mouse lung cancer, PAMM-134Z. In each PCR array, relative expression of 84 target genes and 12 actin housekeeping genes was determined according to the comparative Ct method. qRT-PCR assays were performed to validate the amplification efficiencies of the target genes, and the $2^{-\Delta\Delta Ct}$ method was used to calculate the relative gene expression (presented as fold change) [8]. A change greater that 1.5-fold indicates upregulation, and a change of less than 0.5-fold indicates downregulation. GAPDH was used as a control. Divergent primers were designed and synthesized by Invitrogen Custom Oligo Service (Invitrogen, USA). The primer sequences of objective genes and GAPDH are shown in Table 1.

2.4. Flow cytometry analysis

5 ml of peripheral blood was collected, and the serum was separated by centrifugation after 2 h. According to the manufacturer's instructions, the serum levels of cytokines (IFN-γ, IL-4, and IL-17A) were detected by using a cytometric bead array (CBA) mouse Th1/Th2/Th17 cytokine kit (BD Biosciences). The standards of each cytokine were prepared, the cytokine capture beads were freshly mixed by equal quantity. The mixed beads (50 μL) were incubated with cytokine standard dilutions (50 μL) and Phycoerythrin (PE) Detection Reagent (50 μL) at room temperature for 2 h. The beads were centrifuged (200*g* for 5 min) and resuspended in 120 μL of wash buffer. The results were obtained by using an Accuri C6 flow cytometer (BD Biosciences, San Jose, CA, USA).

Table 1
Primer sequences for qRT-PCR analysis.

Gene	Primer sequences	Product (bp)
GAPDH	Forward: 5′-GGGTCCCAGCTTAGGTTCAT-3′ Reverse: 5′-CATTCTCGGCCTTGACTGTG-3′	248
Clca3	Forward: 5′-CGTTGATCTCAGGGACCACT-3′ Reverse: 5′-GGGAGTTGAGTCTTCGGGAA-3′	250
Prg2	Forward: 5′-ATGGGTGACTCTGGATGCAA-3′ Reverse: 5′-GATCCTGCCTCCAATCCAGA-3′	195
Areg	Forward: 5′-CTTTGGTGAACGGTGTGGAG-3′ Reverse: 5′-TCGTTTCCAAAGGTGCACTG-3′	160
Il13	Forward: 5′-ACTGCGTGATCCTGAGACAA-3′ Reverse: 5′-GCTTCCGTCCAGTTCAAAGG-3′	196
Agr2	Forward: 5′-CCTGCTTCTTGTGGCCATTT-3′ Reverse: 5′-GGGTCTGTTGCTTGTCTTGG-3′	187
Col11a1	Forward: 5′-TCCTGGAACCATGCTGATGT-3′ Reverse: 5′-ATCCCCACTCTCACCTTTGG-3′	208
Sostdc1	Forward: 5′-TGGAGGCAGGCATTTCAGTA-3′ Reverse: 5′-TCCGGCTCCAGTACTTTGTT-3′	212
Ccl1	Forward: 5′-AGCATGCTTACGGTCTCCAA-3′ Reverse: 5′-CGTTTTGTTAGTTGAGGCGC-3′	168
Cxcl11	Forward: 5′-AACAGGAAGGTCACAGCCAT-3′ Reverse: 5′-ACTTCAACTTTGTCGCAGCC-3′	182
Tnfsf4	Forward: 5′-CTGGAAAACGGATCAAGGCC-3′ Reverse: 5′-GCCCATCCTCACATCTGGTA-3′	184

2.5. Haematoxylin and eosin (H&E) staining

All samples were immersed in the haematoxylin (Surgipath, Richmond, USA) for 10 min, and decolorized with 70% ethanol containing 1% hydrochloric acid. After washing, these sections were then placed in 0.1% eosin (Surgipath) for 5 min, and dehydrated in 80%, 90%, and 100% ethanol. After washing, these sections were treated with Oil Red O solution (Sigma-Aldrich, USA) for 10 min. The sections were stained with 1% Picrosirius Red (PSR, Sigma–Aldrich, India) for 10 min, and then dehydrated by using ethanol.

2.6. Statistical analysis

The manufacturer's online tool was used to analyze the array data. Other data were analyzed using Student's t-test and analysis of variance (ANOVA) using GraphPad Prism 6 software. Each experiment was repeated at least three times. The data are expressed as the mean ± standard deviation (mean ± SD). $P < 0.05$ was considered statistically significant.

3. Results

3.1. The distribution of different air pollutants in the laboratory, Guangzhou, and Foshan

We found that the main pollutant in Guangzhou was PM2.5. However, the main pollutants in Foshan were gaseous pollutants including CO, NO_x and SO_2 (Fig. 1).

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