2nd Session

Experimental studies

(Chairs: Ryoji Yamamoto and Xiaohong Zhao)
Susceptibility of lung injury caused by atmospheric fine particles

Weimin Song, Qiang Cao and Rongfang Jiang
School of Public Health, Fudan University, China
Email: wmsong@shmu.edu.cn

The genetic susceptibility of lung injury caused by PM2.5 was studied by using Affymetrix genechip to check the different gene expression of lung tissue in two kinds of mice (C57BL/6 strain mice, and C3H/He (C3) strain mice) exposed to PM2.5 at a dosage of 10 mg PM2.5 /kg BW by intratracheal instillation. It was found that expression of the genes of CHI3L3, CHI3L4, CXCL2, C4, HC, and CP were up-regulated, and expression of the genes of IGH-6, CAP1, and MTAP2 were down-regulated in lung tissues of kinds mice exposed to PM2.5. There were a different expression both in up-regulated genes and down-regulated genes of lungs between two kinds mice exposed to PM2.5. Compared with gene expression in C3H/He, the expression of up-regulated genes in C57BL/6 was obviously higher and the expression of down-regulated genes was obviously lower. Further analysis suggested that most up-regulated genes were related with immune inflammatory response, chemokine activity, cytokine activity and complement activation, while most down-regulated genes were related with positive regulation of endocytosis, cell migration, cell morphogenesis, synthesis and metabolism of nuclei acid and microtubule bundle formation and depolymerization. According to the results, three different signaling pathways between B6 mice and C3 mice were discovered, which included inflammatory response pathway, matrix metalloproteinases pathway and classical complement activation.

In order to prove influence of existed cardiopulmonary disease to the effects of PM2.5 on health, both rat models of chronic bronchitis and Spontaneously Hypertensive Rats (SHR) were used. According to the results the concentration of albumin (ALB), lactate dehydrogenase (LDH), alkaline phosphatase (AKP) and malonaldehyde (MDA) in BALF of rats with chronic bronchitis was higher than those in rats without chronic bronchitis, whereas the glutathione (GSH) was lower although both rats exposed to same dosage of PM2.5. In the rat model of hypertension, not only the gene expression of MDA, TNF-α, IL-1β, MIP-2, CD44 and OPN related with inflammation, but also the expression of CC16, SP-A and HO-1 related with anti-inflammation were increased, whereas the expression of SOD and GSH related with anti-oxidative damage were decreased in lung tissue of rats with both hypertension and none-hypertension exposed to fine particles. Compared with normal rats, the expressions of those genes in rats with hypertension were significantly higher except SOD and GSH.

In summary, both genetic and cardiopulmonary diseases were susceptible factors of lung injury caused by fine particles.
Study on the toxicological effects of extracts of fine particles on vascular endothelial cells

Jinzhuo Zhao, Weimin Song and Rongfang Jiang
School of Public Health, Fudan University, China

In order to illustrate toxicological mechanism of the components of fine particles on vascular endothelial cells, EC-304 was exposed to organic extracts, soluble extracts and insoluble extracts of fine particles respectively with or without the atorvastatin intervention. Oxidative stress, inflammation and endothelial dysfunction were observed for toxicological effects of the extracts of fine particles. In the culture system EC-304 was exposed to three kinds extracts respectively with the concentration of 400 μg/mL and astorvastatin with concentrations of 0μmol/L, 0.1μmol/L, 1μmol/L and 10μmol/L respectively.

The results showed that in comparison with no atorvastatin intervention, the levels of ROS, MDA and LDH in EC-304 cells exposed to different extracts of fine particles and atorvastatin were obviously lower. In the contrast, cell activities, SOD and NO levels in EC-304 cells with atorvastatin intervention were significantly higher than those without atorvastatin intervention. Furthermore, the mRNA expression of IL-4, IL-6 TNF-α, ET-1, P-selectin, TGF-β1 and fas in EC-304 cells exposed to particles with atorvastatin intervention were obviously lower than those without atorvastatin intervention.

The study suggested that oxidative stress, inflammation and endothelial dysfunction were the potential mechanisms of cardiovascular injuries caused by fine particles. Statin showed the benefit effects to improve endothelial function and inhibit inflammation and modulate immune function and reduce oxidation caused by ambient fine particles.
Research advances on sulfur dioxide toxicology in China
Quan-xi Zhang and Zi-qiang Meng
Institute of Environmental Medicine and Toxicology, Shanxi University, Taiyuan 030006, China

The toxicological and physiological effects of sulfur dioxide (SO2) have been investigated mainly in our laboratories for more than 20 years.

First, we investigated oxidative damage and cellular ultrastructure damage effects of SO2 on various organs of mice and rats. These results lead to two conclusions: (1) SO2 is a systemic oxidative damage and cellular ultrastructure damage agent. (2) The oxidative damage and cellular ultrastructure damage produced by SO2 inhalation might influence or promote progression or occurrence of some disease states of various organs, not only to respiratory system.

Second, gene expression profiles of the lungs of Wistar rats short-term and long-term exposed to SO2 were studied by Affymetrix GeneChip (RAE230A) analysis. It is suggested that: (1) a notable feature of the gene expression profile was the decreased expression of genes related to oxidative phosphorylation in lungs of rats short-term exposed to SO2, which shows high-dose short-term exposed to SO2 may cause the deterioration of mitochondrial functions; (2) discriminating genes in lungs of rats long-term exposed to SO2 included those involved in fatty acid metabolism, immune, inflammatory, oxidative stress, oncogene, tumor suppresser and extracellular matrix. The mechanism of low-dose long-term exposed to SO2 is more complex.

Third, the mRNA and protein levels of apoptosis-related genes (p53, bax and bcl-2), cytochrome P450 (CYP1A1 and CYP1A2), proto-oncogenes (c-fos, c-jun, and c-myc) and tumor suppressor genes (p53, p16, and Rb) were analyzed in lungs and livers, respectively. These results lead to the conclusions that SO2 exposure can change the expression of these genes. Elucidating the expression patterns of those factors after SO2 inhalation may be critical to our understanding mechanisms

Fourth, the effects of SO2 derivatives (sulfite and bisulfite) on gene expressions of some asthma-related genes in human bronchial epithelial cells (BEP2D) were investigated. It was suggested that SO2 derivatives could increase the expressions of EGF, EGFR, ICAM-1 and COX-2 on the transcription and translation levels in BEP2D cells, and result in mucus over-production and inflammation responses. This might be one of the possible mechanisms that SO2 aggravates asthma disease.

Fifth, sister-chromatid exchanges and micronuclei caused by SO2 in various cells were also firstly studied in our laboratories in 1980s, we found SO2 and its derivatives can cause the increases of chromosome abberations, sister-chromatid exchanges and micronuclei in human blood lymphocytes and other mammalian cells.

Recently, we find endogenous gaseous SO2 is a gasotransmitter, the mechanism of SO2-induced vasorelaxation was shown to be endothelium-independent at high concentrations and endothelium-dependent at physiological and low concentrations, in part, involved the contribution of BKCa and KATP channels as well as possible alterations in Ca-influx and release pathways.

Acknowledgements
This research was supported by the National Natural Science Foundation of China.
The vasorelaxant effect of endogenous gaseous sulfur dioxide on aortic rings and its mechanism: A comparison with sulfite effects and SO2 biosynthesis and regulation

Zi-qiang Meng*, Jun-ling Li, Quan-xi Zhang, Wei-ming Bai, Zhen-hua Yang, Fa-qi Wang and Ying Zhao

Institute of Environmental Medicine and Toxicology, Shanxi University, Taiyuan 030006, China

To study physiological role of endogenous gaseous sulfur dioxide (SO2) on vascular contractility and its mechanisms, isolated rat thoracic aortic rings were exposed directly to SO2 gas. And a comparison study between vasorelaxation effects caused by gaseous SO2 and its derivatives sodium sulfite and bisulfite (about 3:1, molar ratio, pH 7.0) was carried out in order to explore inactivation pathway of SO2 as a gas transmitter. At one time SO2 physiological concentration in rat blood plasma and thoracic aortic tissue was measured, and SO2 biosynthesis and regulation in vascular tissues were studied. Results showed that 1) vasorelaxation effect was caused by gaseous SO2 (1~2000µmol·L-1) in a dose-dependent manner, the effect by gaseous SO2 was much greater than that of sulfite and bisulfite, suggesting the latters were metabolic products in inactivity process of SO2 as a gas transmitter; 2) Mean concentrations of endogenous SO2 in rat vascular tissues and blood plasma were (127.76±31.34) and (16.77±8.24)µmol·L-1, respectively, implying SO2 concentration in the vascular tissues was very high; 3) Endogenous SO2 could be synthesized in both the vascular endothelial and smooth muscle cells, but mainly in the vascular endothelial cells; 4) Acetylcholine (Ach) could increase levels of endogenous SO2 in rat vascular tissues both in vivo and in vitro, and also enhance production of endogenous SO2 in both cultured rat vascular endothelial and smooth muscle cells; However, noradrenaline (NE) inhibited SO2 generation in the vascular tissues and cells. These results led to the conclusions: 1) SO2 derivatives sulfite and sulfate were metabolic products in the inactivation process of SO2 gas transmitter; The inactivation pathway was “gaseous SO2 → sulfite/bisulfite → sulfate”; The physiological role of endogenous SO2 was different from that of its derivatives sulfite/bisulfite; 2) That biological tissues or organs were treated using SO2 gas or its solution (in normal saline) was a new research model for SO2 physiology in vivo and in vitro; 3) SO2 concentration in rat vascular tissues was high and endogenous SO2 was generated mainly in the vascular endothelial cells; 4) Biological synthesis of SO2 could be regulated by endogenous substances such as Ach and NE. Besides, it was discussed that mixture of sodium sulfite and bisulfite could not be used as “SO2 donor” to treat biological tissues.

Keywords: sulfur dioxide; sulfur dioxide derivatives; sulfite; physiological concentration; regulation; gas transmitter; vasorelaxation; SO2 donor

Acknowledgements
This research was supported by the National Natural Science Foundation of China.
The study of the damage and its mechanisms induced by PM$_{2.5}$ from Beijing urban in A549 cells

Xiaohong Zhao, Xuan Liu, Guifang Zu and Hui Peng

Dept. of Biology, College of Applied Arts and Sciences, Beijing Union University, Beijing, China, 100191.

Objective: Many epidemiologic studies have shown that PM2.5 plays an important role in increasingly respiratory diseases. However, the exact toxicological mechanisms of PM2.5 still remain unresolved. To investigate the toxicological mechanisms of PM2.5 on A549 cell, cell proliferation, DNA strand breakage, the activity and expression of NF-$\kappa$B were studied.

Methods: PM2.5 was collected in heating period in the urban area of Beijing and was extracted by water ultrasonic method. Cytotoxicity of PM2.5 was measured by MTT assay. The degree of DNA strand breaks was measured by Comet assay. The activity of NF-$\kappa$B was measured by EMSA and ELISA binding assay. The levels of NO expressions were determined by using the nitrate reductase method. The NOS activity was detected by Colorimetry. Western blot method was used to detect the expression of NF-$\kappa$B in A549 nuclear. Results: The results showed that (1) PM2.5 induced A549 cell proliferation at low doses, but inhibited cell proliferation at high doses, and the cell livabilities were decreased with the increased concentrations. (2) DNA single- and double-strand breaks were obviously increased in A549 cells exposed to PM2.5, with the significant dose-response relationship ($r=0.97$, $p<0.01$), and double-strand damage was more serious than single-strand damage. (3) The activity of NF-$\kappa$B were increased in cell nuclear, but decreased in cell plasma after exposed to PM2.5 with the significant dose-response relationship ($r=0.98$, $p<0.01$), which suggested that activated NF-$\kappa$B can move into the cell nuclear. Compared with control, PM2.5 (100mg/L) could cause the high expression of NF-kB in A549 nucleus, with statistically significant difference ($p <0.05$). (4) The levels of NO were increased in A549 cells exposed PM2.5 for 24h, but the change of NOS activity didn’t be found. (5) At certain concentration, PM2.5 induced significantly high expression of NF-kB in A549 nuclear. Conclusion: PM2.5 inhibited cell proliferation in A549, and induced oxidative and inflammatory damage after treated with PM2.5 for 24h.

Keywords: PM2.5; A549 cell; cell proliferation; comet assay; inflammatory cytokines; NO; NF-$\kappa$B
Application of a new method using PIXE to analyze fluoride and multi-elements in environmental samples

Shiro Sakurai
Department of Environmental Science, School of Social Information Studies, Otsuma Women's University, 2-7-1 Karakida, Tama, Tokyo, 206-8540, Japan
sakura@otsuma.ac.jp

We recently developed a three-detector measuring system making use of a pure-Ge detector combined with two Si(Li) detectors. The efficiency curve of the pure-Ge detector was determined as relative efficiencies to those of the existing Si(Li) detectors and accuracy was confirmed by analyzing a few samples whose elemental concentrations were known. It was found that detection of fluorine becomes possible by analyzing prompt $\gamma$-rays and the detection limit was found to be less than 0.1 ppm for water samples. In this study, a method of quantitative analysis of fluorine has been established in order to investigate environmental contamination by fluorine. This method is based on the fact that both characteristic x-rays from many elements and 110 keV prompt $\gamma$-rays from fluorine can be detected in the same spectrum. The present method is applied to analyses of a few environmental samples such as tea leaves, feed for domestic animals and human bone and teeth. The results are consistent with those obtained by other methods and it is found that the present method is quite useful and convenient for epidemiological studies on regional pollution by fluorine.

China has a lot of dental or skeletal fluorosis due to high fluoride intake from drinking water and food.

We have applied to determine concentrations of fluorine and multi elements in shark teeth collected in the south of Japan as a kind of example. As a result, it was confirmed that the sample preparation method, which was established for the biological samples, is applicable to the shark teeth samples and elemental concentration was obtained in good accuracy and reproducibility. Moreover, we clarified that the shark teeth is composed of Fluorapatite by the combination with X-ray Diffraction. Fluorine concentration is found to be 5500 $\mu$g/g in the shark teeth.

Keywords: Fluorine, Multi-elements, PIXE