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European Journal of Radiology 58 (2006) 266–272

EJR
EUROPEAN JOURNAL OF RADIOLOGY

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Synchrotron microradiography study on acute lung injury of mouse caused by $PM_{2.5}$ aerosols

Yongpeng Tong [a], Guilin Zhang [a,*], Yan Li [a], Mingguan Tan [a], Wei Wang [a], Jianmin Chen [a], Yeukuang Hwu [b], Pei-Chebg Hsu [b], Jung Ho Je [c], Giorgio Margaritondo [d], Weiming Song [e], Rongfang Jiang [e], Zhihai Jiang [e]

[a] *Shanghai Institute of Applied Physics, Chinese Academy of Sciences, Shanghai 201800, China*
[b] *Institute of Physics, Academia Sinica, Nankang, Taipei*
[c] *Department of Material Science and Engineering, Pohang University of Science and Technology, Pohang, Korea*
[d] *Faculté des sciences de base, CH-1015 Lausanne, Ecole Polytechnique Fédérale de Lausanne (EPFL), Switzerland*
[e] *School of Public Health, Fudan University, Shanghai 200032, China*

Received 28 June 2005; received in revised form 28 November 2005; accepted 29 November 2005

Abstract

In order to investigate $FeSO_4$, $ZnSO_4$ (the two of main metal compositions of Shanghai $PM_{2.5}$ (particle matter with those aerodynamical diameter <2.5 μm)) effects on acute lung injury, six solutions contained $PM_{2.5}$ aerosol particles, $FeSO_4$, $ZnSO_4$ and their mixtures were instilled intratracheally into mouse lungs for experiment. By 2 days after instillation, the live mice were checked in vivo by synchrotron refractive index microradiography. In addition after extracted and examined by dissection, the right lobes of lung were fixed by formalin, then imaged by synchrotron microradiography again. Corresponding parts of those lung tissues were embedded in paraffin for histopathologic study. The synchrotron X-ray microradiographs of live mouse lung showed different lung texture changes after instilled with different toxic solutions. Hemorrhage points in lung were observed more from those mice instilled by $FeSO_4$ contained toxin solutions groups. Bronchial epithelial hyperplasia can be observed in $ZnSO_4$ contained solution-instilled groups from histopathologic analysis. It was found that the acute lung injury of mice caused by solution of $PM_{2.5} + FeSO_4 + ZnSO_4$ was more serious than other toxin solutions. Results suggested that $FeSO_4$ mainly induced hemorrhage and $ZnSO_4$ mainly induced inflammation and bronchiolar epithelial hyperplasia in the early toxicological effects of $PM_{2.5}$.

Keywords: Synchrotron microradiography; Lung injury; $FeSO_4$; $ZnSO_4$; $PM_{2.5}$

1. Introduction

The increasing mortality and morbidity due to cardiopulmonary complications are attributed to elevated concentration levels of ambient particulate matters, in particular, of small inhalable particles [1]. Therefore, it is essential to understand in detail the mortality mechanism induced by such fine particles. Many reports suggest that $PM_{2.5}$ induce reactive oxygen species (ROS) and inflammatory mediators, resulting in vascular permeability changes, airway constriction and tissue injury [2,3]. The transition metal ions and peroxides in aerosols can induce free radicals and cause both cytotoxicity and a strong oxidation response [4]. Based on the previous report [5], it is found that the main acute effects of PM are due to soluble ions. Shanghai as one of most quickly developing cities in the world in economics, its energy exhausts are also raised quickly. The Chinese biggest iron plant—Baoshan steel plant and electronic power plants make two of the main contributions to $PM_{2.5}$ in Shanghai. Fe and Zn are the two main transition elements in $PM_{2.5}$ and high SO_2 (from coal burning) even make those aerosols more toxic to our health [3]. Those soluble metal constituents of residual oil fly ash (ROFA) particles can enhance the sensitization

* Corresponding author. Tel.: +86 21 59553998; fax: +86 21 59553021.
E-mail address: glzhang@sinap.ac.cn (G. Zhang).

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doi:10.1016/j.ejrad.2005.11.035

of lung injury [6]. As $PM_{2.5}$ collected from the industrial city—Shanghai contains relatively higher transition elements for instance, Fe, Zn, etc. [7], and higher sulfates [8], it is important to study the effects of the main transition compositions $FeSO_4$ and $ZnSO_4$ in $PM_{2.5}$ induced acute pneumonia process.

Many studies related to pneumonia and cytotoxicity were carried out on the histopathological examination of lung section [9–11]. Usually, in those previous work of toxicological studies on tissues, performed by optical microscopy and the scanning proton microprobe [7], only thin tissue samples (<50 μm) excised from killed rats [10,12] were used on some respiratory function tests [13] and on analyzing the structural changes [10]. Our present work proves that such critical limitation can be lifted by using edge-enhanced microradiographs with high energy of X-rays which can penetrate a mouse. The high intensity of X-rays makes it possible to achieve high resolution in a short time, for instance, 3 ms or less, as what required for imaging a live mouse without observed damage [14,15]. Here, it is important to use this method in vivo to monitor acute pulmonary toxicity after the mouse intratracheally instilled as the $PM_{2.5}$ toxic effect is a developing process [16]. Combining those studied results of the transition element Fe, Zn effects on lung epithelial cultured cells [17] with this studying result on lung tissue structure, it may understand more about Fe, Zn toxic effects in the industrial city $PM_{2.5}$.

2. Experimental methods

2.1. Aerosols sampling

$PM_{2.5}$ samples were collected by a stacked filter air sampler at the Baoshan area which is one of the industrial districts in Shanghai. The $PM_{2.5}$ aerosols were collected on Teflon filters at 6.5 m above ground at a flow rate of 78 l/min. Each sample required ∼360 h by a middle flux air sampler and all aerosol samples were collected during the period of September–November 2003.

2.2. Elemental analysis

The elemental analysis for $PM_{2.5}$ was carried out by a VG X7 ICP-MS instrument (Thermo electron corporation) and at least 16 elements were found.

2.3. Toxin sampling

Several films contained $PM_{2.5}$ (total 200 mg) were first immersed into physiological saline, then the particles were disinfected by ultrasound for 1 h at about 50 °C. During this process, it was found that most of bacteria detected by standard bacteria culture method were killed (less than 3 ml^{-1}). This solution was kept at low temperature (0 °C). Six solutions (pH ∼ 5.3), i.e. $PM_{2.5}$ aerosol solution 25 mg/ml, $FeSO_4$ solution 15 mg/ml, $ZnSO_4$ solution 15 mg/ml and mixed solutions of $PM_{2.5}$ 25 mg/ml + $FeSO_4$ 15 mg/ml and $PM_{2.5}$ 25 mg/ml + $FeSO_4$ 15 mg/ml + $ZnSO_4$ 15 mg/ml and saline were prepared for mice instillation.

Animal grouping and instillation: male KP600 CD-1 mice, weighing 22–26 g, were obtained from the Experimental Animal Center of Pohang University of Science and Technology, Pohang, Korea. Total of 36 mice were grouped randomly into 6 groups on an average and each group (6 mice) was, respectively, instilled intratracheally with each test materials: saline, Fe SO_4, $ZnSO_4$, $PM_{2.5}$, $PM_{2.5}$ + $FeSO_4$, $PM_{2.5}$ + $FeSO_4$ + $ZnSO_4$ solutions, respectively, 0.04 ml solution per mouse was instilled every day. The dose and time point used here were selected based on pre-test by histopathological examination of the lung tissues excised from the killed mouse. By 48 h after twice instillations (0 and 24 h), the right lung of live mice were observed by synchrotron X-ray imaging. Then, the mice were anesthetized with an intraperitoneal injection of 10 mg (450 mg/kg) chloral hydrate (0.2 ml of 5% Sigma chemical). After they were anesthetized, the mice were killed by cutting neck, then dissected and observed by eyes immediately. This animal experimental process is permitted by Law and Ethics Committee. Finally, the right lung tissue was fixed by formalin for further histopathological analysis.

2.4. Study of the irradiation influence on lung tissue during synchrotron X-ray imaging

Ten mice were taken images at their chest position for a different radiation time (3–15 ms). After dissecting those mice it was found that there were no significant changes for the lung tissue compared with those control mice without synchrotron X-ray irradiation by histopathological analysis. In addition action and behavior of mice subjected to irradiation also did not show any difference with those of the control mice. Results showed that the exposure by irradiation during the imaging within a time range of 3–15 ms did not induce significant extra effects to the mice. Finally, 3 ms were chosen for performing synchrotron X-ray imaging.

2.5. Lung tissue sampling and analysis

Part of the right lobe of the lung was fixed in formalin, processed and embedded in paraffin. Lung pathological sections of right lobe with a thickness of 5 μm were observed by optical microscope in order to compare with the corresponding the imaging of live mice taken by synchrotron X-rays. More than 10 lung pathological sections of each mouse lung tissue have been analyzed by optical microscope to outline toxic effects. Although it is difficult to get the section to be analyzed by optical microscope in the same area where the microradiography was imaged, some typical poisoning characters of lung tissue can be compared. The right lobe tissue was selected for experiment mainly due to the imaging process of right lobe is less affected by heart beating.

2.6. Phase contrast microradiography

As is well known, coherence light passing a object can produce Fresnel diffraction. Like the classic Fresnel edge diffraction X-ray beam with sufficient coherence can produce edge enhancement by the phase contrast mechanism in radiological images [18]. Highly coherent synchrotron X-ray sources provide better image quality than conventional radiology and much deeper penetration (cm) than optical microscope. There also exists other contrast mechanism based on the refraction in X-rays. As commonly used in phase contrast optical microscope, at the edge between two areas with different refractive indexes, the different deviations of the light beams by the two sides create typical white–dark "fringes" that enhance the edge visibility. As is well known, the lung consists of a lot of alveoli besides other tissue, in the alveoli it is full of air, the refraction index between air and alveolar wall is large different. Therefore, the imaging, based on it might be a very efficient way to study the pneumonia in microstructure. In this experiment, the parameter which is a distance between sample and detector was chosen such that the refraction phase contrast effect played a role, whereas other types of phase contrast effects were washed out [15]. Edge enhancement effects were in fact clearly visible even without monochrometization [19] by this method.

The microradiography measurements were performed at beamlines 5C1 and 7B2 at Pohang Light Source (PLS), Korea. The X-rays, emitted by an electron storage ring with electron energy of 2.5 GeV and a typical beam current of 150 mA, went through two beryllium windows and reached the specimen. Polychromatic synchrotron X-rays were used with an energy range of 4–15 keV. A set of shutters made of silicon slabs with different thickness was used as an attenuator to control the total X-ray flux and the photon energy distribution. After passing through the specimen, the X-rays reached a thin $CdWO_4$ scintillator crystal producing a visible image that was captured by a CCD camera. The size of the beam spot is 5 mm × 5 mm. The typical spatial resolution is <2 μm far from the limit resolution of the experimental system, ∼0.3 μm [15]. The exposure time for each microradiograph was 3 ms. The live mouse fixed in a shelf or the lung tissue sample was placed ∼150 mm from the scintillator to optimize the detection of the phase contrast effects. In contrast with the conventional X-ray absorption images of soft tissues this method can achieve a dramatic improvement for the lung microradiograph. The right lung tissues of each mouse were imaged.

3. Results and discussion

3.1. Elemental contents of $PM_{2.5}$

The $PM_{2.5}$ solution used for instillation was analyzed, as already mentioned, by X7 ICP-MS. The results are shown in Table 1. It is clear that the concentration of transition elements (Fe, Zn) is much higher than others (Cr, Ni, Cu, Pb, etc.). It can also be found that higher S in Shanghai $PM_{2.5}$. Furthermore, Fe sulfate is one of main compositions in Fe contained $PM_{2.5}$ in the previous studies [8,20]. The higher content of transition metal Fe, Zn sulfates in Shanghai $PM_{2.5}$ may be play important roles in lung diseases as Fe and Zn is toxic and more easily induces mouse pneumonia [4,9,17]. So it is reasonable to use Fe, Zn sulfates for instillation experiments to study their toxic effects in $PM_{2.5}$.

3.2. Microradiography

X-ray imaging experiments in vivo were performed to determine the lung tissue structure changes caused by $PM_{2.5}$ instillation. Typical images are shown in Fig. 1. As is known the size of lung alveolus is about several dozens of micrometers, it is impossible to be observed by conventional X-ray imaging as showed in Fig. 2 which are two photographs of mouse taken by conventional X-rays after instillation of saline and the solution of $PM_{2.5} + FeSO_4 + ZnSO_4$, respectively. Those two photos only show contrast area in different intensity of black and white color without any lung tissue structure, and no difference between two photos could be found. By compared study, it is found that synchrotron X-ray phase contrast imaging shows much higher resolution. In those micrographs, traces of the lung alveolus could be observed and some changes in the lung tissue and alveolus structure of mice after exposed to toxin solutions could be found. Dark hemorrhage spots with ∼0.5 mm as indicated by arrows and un-uniformity of appearance of lung texture were found nearly for every exposed group but not in control group. Statistically those images of $FeSO_4$ contained groups showed more hemorrhage spots, whereas $ZnSO_4$ contained groups showed more un-uniformity of appearance of lung texture.

The image of $PM_{2.5} + FeSO_4 + ZnSO_4$ showed the biggest change in lung texture structure. In addition the clearances of lung lobes could be identified in the exposed group except the control group. So we may suggest that the clearances of lung lobes are bigger in the exposed groups than that of control group. Air particles induced lung hemorrhage, inflammation has been widely reported and the hemorrhage was considered as early stage of lung injury [21]. Some transition metal ions

Table 1
The concentration of elements in $PM_{2.5}$ aerosols (μg/mg)

Elements	Content	Elements	Content
S	60.6 ± 0.2	Zn	8.87 ± 0.06
K	30.9 ± 0.2	Mo	0.06 ± 0.01
Cd	0.08 ± 0.01	Ni	0.13 ± 0.01
Cr	0.22 ± 0.02	Pb	4.19 ± 0.04
Cu	0.15 ± 0.02	Ti	2.17 ± 0.03
Fe	26.1 ± 0.1	As	0.36 ± 0.01
Na	11.9 ± 0.1	Se	0.16 ± 0.01
Mn	1.78 ± 0.02	Sb	0.20 ± 0.01

N (number of samples) is 4.

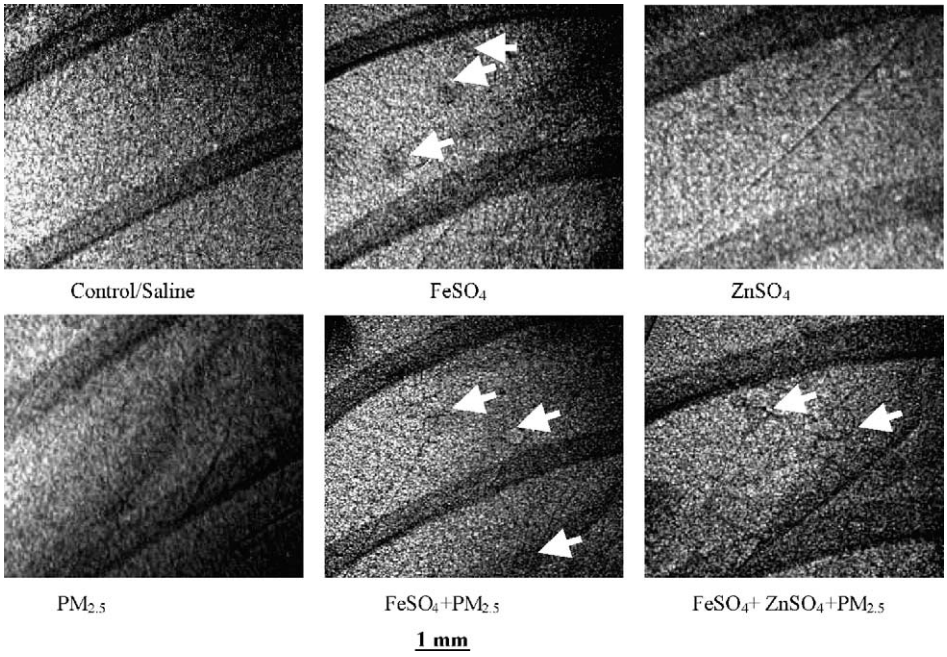

Fig. 1. Synchrotron microradiographs of right lung position for different instilled live mice groups by 2 days instillation (acquired in vivo).

(Ni and V) with particles induced lung hemorrhage was also reported [22,23]. It is well known that those transition metal ions (Fe, etc.) can induce free radical by the Fenton's reaction. Free radicals may cause those small blood vessels broken in the early stage of lung injury process as a free radical scavenger can inhibit cerebral hemorrhage [24].

The typical micrographs of fixed lung tissues by synchrotron microradiographs are showed in Fig. 3. Obviously it can be observed that the alveolus structures of all toxin-exposed groups are turned to be more aggregated than those of the control group. The inhaled functions may be inhibited in toxin-instilled groups. Especially, it similar to Fig. 1, those images from $ZnSO_4$ groups showed more changes in lung texture. This result may suggest that the $ZnSO_4$ can induce more serious inflammation which destroy the tissue structure as Zn can stimulate PBMC (monocytes) in a dose-dependent manner to release inflammation factors IL-1, IL-6, tumor necrosis factor (TNF)-α and IFN-γ [25].

Hemorrhage points were observed for the dissected lung tissues in some parts of right lung by microscope in the groups of mice instilled by toxin solution. The observed hemorrhage positions are consistent with the results observed by X-ray microradiology.

3.3. Histopathology of lung injury induced by $PM_{2.5}$ and other toxin solutions

The typical histopathological changes of lungs with thickness of 5 μm in different mouse groups are shown in Fig. 4. The histopathological results showed that hemorrhage was also found in $FeSO_4$-exposed group as indicated by arrows, bronchial epithelial hyperplasia was found in $ZnSO_4$-exposed group (at the arrow area) and the complicated inflammation effects were found in $PM_{2.5}$-exposed group. Compared to the lung injury in $PM_{2.5}$ group, the solution contained Fe in $PM_{2.5} + FeSO_4$ group induced more effects

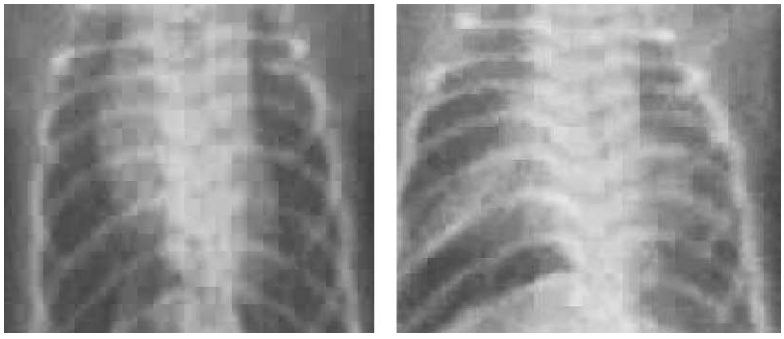

Fig. 2. The radiographs were taken by conventional X-rays imaging after a mouse was instilled the solution of saline (left) and $PM_{2.5} + FeSO_4 + ZnSO_4$ (right) by 2 days after instillation, respectively.

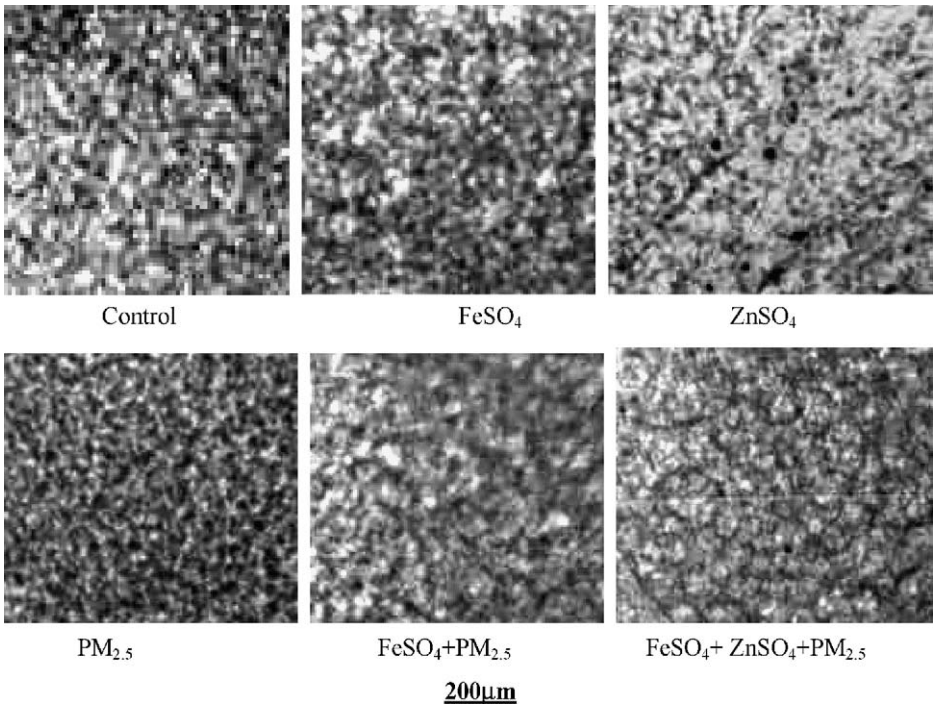

Fig. 3. Synchrotron microradiographs of right lobe of fixed lung tissue for different instilled groups by 2 days after instillation.

of hemorrhage, and the solution contained Fe, Zn and $PM_{2.5}$ induced serious inflammation effects such as thickened alveolar septae and pus in bronchia as well as bronchial epidermal cell hyperplasia in $PM_{2.5} + FeSO_4 + ZnSO_4$ group. In addition numerous neutrophils, lymphocytes and macrophage were found in the areas of inflammatory cell infiltrates for the mice instilled by toxin solution. These results suggest that the effects of $PM_{2.5}$ on lungs may be different for contained different inorganic compositions in $PM_{2.5}$. By compared study of the three kinds of micrographs, i.e. synchrotron X-ray microradiograph on live mice and fixed tissues and histopathological observation

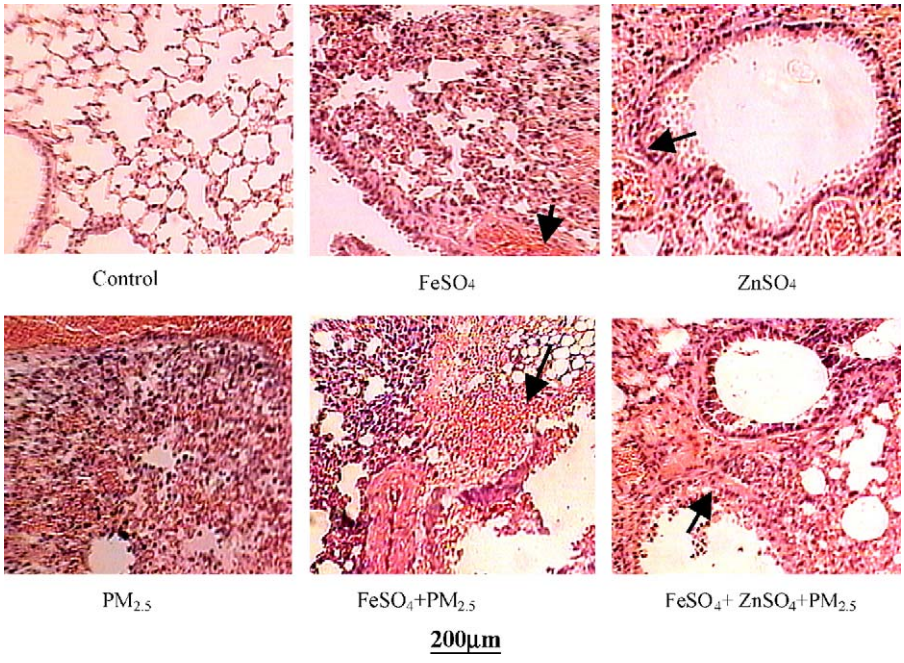

Fig. 4. Typical histopathological figures of right lobe of fixed lung tissue sections for different instilled groups by 2 days after instillation.

to the corresponding slices, the complemented information can be gotten. The histopathological observation can help to understand those images taken by synchrotron X-ray microradiograph.

Many authors [26] argued that PM can cause thickened bronchus walls and strongly reactive oxygen species at the early stage of a lung tissue lesion. It was also reported that long-term exposure to high levels of ambient particulate pollutants is associated with remodeling of small airways. This process can cause chronic airflow obstruction [27]; PM-induced changes in the lung function (gas exchange, etc.) are also associated with asthma [28]. It is well known that PM exposure adversely affects lung functions and worsens lung injuries (lung hemorrhage, etc.). Oxidative stress is expected at the PM surface and can be augmented by oxidants generated by recruited inflammatory leukocytes. Our present results suggest that Fe and Zn ions combined together in $PM_{2.5}$ play an important role in the inducing pulmonary toxicity, they may help $PM_{2.5}$ to enhance this toxic process.

As synchrotron microradiology could directly detect the precursor structural changes of lung tissue, in particular lung tissue hemorrhage process which is one of the main causes of death, the power of synchrotron microradiography in study of microscopic lung texture structural changes in biological specimens can be observed. Microradiographs can be taken in vivo on a live mouse with high time resolution to monitor the lung poisoning process.

4. Conclusion

In conclusion, the synchrotron X-ray microradiographs observed in vivo show much high resolution which can monitor mouse hemorrhage process. By that un-uniformity of appearance of lung texture and hemorrhage spots with a size of <0.5 mm can be found for groups exposed to toxin solutions but not in saline solution. The hemorrhage spots and hemorrhage lines appeared in the group of mice instilled by solution of $PM_{2.5} + FeSO_4 + ZnSO_4$ were more than the ones in other groups of mice. $FeSO_4$ induced more hemorrhage and $ZnSO_4$ induced more inflammation and bronchial epithelial hyperplasia in toxicologic effects of the industrial particle $PM_{2.5}$. Fe and Zn can enhance $PM_{2.5}$ toxicologic effects.

The synchrotron microradiography is a good imaging method to study alveolus structure and pulmonary injury, especially, it can be performed in vivo.

Acknowledgements

This work was supported by grants from Shanghai Nature Science Foundation of China (03ZR14111), a Major Project of Knowledge Innovation Program of Chinese Academy of Sciences (Contract No. KJCXZ-SW-No1) and a Major Project of the National Natural Science Foundation of China (Grant No. 10496182).

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