Upregulated Expression of Secretory Leukocyte Protease Inhibitor in Lung by Inhalation of High Concentration of Sulfur Dioxide

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To the Editor: Sulfur dioxide (SO₂) is one of the main air pollutants, which is formed when sulfur-containing fuel is burned. In some special situations, people may be exposed to high concentration of SO₂ such as an accident of SO₂ tank leakage, mine blast, smoke of gunpowder in a war, and smoke of volcanic eruption, which may be harmful to the persons who are exposed to it. Many studies have indicated that SO₂ exposure increases morbidity and mortality. SO₂ can not only produce a variety of adverse pulmonary effects, such as bronchitis and airway hyperresponsiveness, but also have harmful effects on other systems and organs. It is associated with increased risk of acute myocardial infarction, and inhalation of SO₂ can cause injury in brain including stroke. The expressions of oncogenes and tumor suppressor genes in lung and liver of rat were affected by exposure to SO₂ and benzo(a)pyrene. The expressions of apoptosis-related genes can be augmented and the apoptosis of the cells was induced in liver, lung, and brain of rats exposed to SO₂.

Secretory leukocyte protease inhibitor (SLPI) is a glycoprotein with a molecular weight of about 11,700. SLPI is present in human mucus secretions and tissues and produced primarily in the epithelial cells lining the respiratory, digestive, and reproductive tracts. Its major physiological function is to inhibit serine proteases, including cathepsin and tryptase, and to protect tissues from excessive protease digestion at the sites of inflammation in vivo. It has antibacterial and antifungal properties in vitro and has been shown to prevent viral infection. SLPI inhibits the expression of inflammatory cytokines such as tumor necrosis factor (TNF)-α, interleukin (IL)-8, and IL-6 via translocation from cytoplasm to nucleus and binding to nuclear factor-kappa B binding sites.

Although SLPI is well characterized at both the gene and protein levels, little is known about the regulation of SLPI expression in the lung. In this study, we investigated whether the expression of SLPI both at mRNA and protein levels in the lungs of rats is influenced by inhalation of high concentration of SO₂.

Animals experiments was approved by the Ethics Committee of General Hospital of Shenyang Military Command. An exposure chamber of SO₂ was designed. The device consisted of SO₂ source, air pump, intake port, SO₂ chamber, and SO₂ detector and some connective tubes and valves. The SO₂ was diluted with fresh air at the intake port of the chamber to yield the desired SO₂ concentrations. The desired SO₂ was delivered to animals via a tube positioned at the upper level of the chamber and distributed homogeneously via a fan in each chamber. The concentration of SO₂ was determined in real-time manner by a SO₂ sensor (JSAS5- SO₂ sensor, Shenzhen Ji-shun-an Technology Co., Ltd., China). The concentration of SO₂ in the chamber was adjusted by opening and closing a valve between intake port and SO₂ chamber according to the quantitative value of the SO₂ sensor.

The Sprague-Dawley rats were divided randomly into two groups with 10 rats in each group. The rats in SO₂ exposed group were placed into the exposure chamber described above and exposed to 6×10⁴ SO₂ for 2 h each day for consecutive 7 days. The rats in control group were exposed to filtered air in another identical chamber for the same period of time. When not being treated, all of the rats had free access to food and water ad libitum. After the SO₂ exposure was finished, the rats of both groups were sacrificed and the sera and bronchoalveolar lavage fluid (BALF) were harvested and restored at −20 °C until detection. The lungs were removed and divided into three parts for reverse transcription-polymerase chain reaction (RT-PCR), Western blot, and histological analysis.

Total RNA was isolated from <100 mg of lung tissue using TRIzol reagent (Invitrogen Life Technologies, USA) according to the manufacturer’s instructions. Quantitative RT-PCR (QRT-PCR) was performed using a sequence detection system (ABI PRISM 7000; Applied Biosystems, Life Technologies, Grand Island, NY, USA), and all reaction components were purchased from the same source (SYBR PrimeScript RT-PCR Kit obtained from TaKaRa Biotechnology). The standard experiments were carried out based on the instructions of the provider. All reactions were performed...
were observed in SO groups. The pathological changes, such as chronic bronchitis, local alveolar hemorrhage, and lymphocytes infiltration, were observed in SO group (d). Scale bar = 100 μm. SO: Sulfur dioxide; SLPI: Secretory leukocyte protease inhibitor.

Figure 1: Effects of high concentration of SO\(_2\) on the SLPI expression in lung of rats (a and b). The gray values of SLPI protein level in control and SO\(_2\) groups. β-actin as the internal control. The histopathological test (HE staining) showed that no abnormality in lungs and tracheas was observed in control group (c), and the pathological changes, such as chronic bronchitis, local alveolar hemorrhage, and lymphocytes infiltration, were observed in SO\(_2\) group (d). Scale bar = 100 μm. SO\(_2\): Sulfur dioxide; SLPI: Secretory leukocyte protease inhibitor.
Air pollution has been paid special attention by the public because it gives rise to many health and environmental problems. SO$_2$ is one of the most frequently exposed air pollutants. Many evidences have demonstrated that some respiratory-tract and cardiopulmonary diseases were caused by exposure to SO$_2$. The researchers have shown inflammatory cell infiltration, mucus cell increase, and mucus hypersecretion in the airways of SO$_2$ exposed rats. However, to the best of our knowledge, little studies focused on the effect of SO$_2$ on the expression and function of SLPI. The present study demonstrated that the expression of SLPI was upregulated both at mRNA and protein levels in the lung tissue of SO$_2$ group. Because SLPI is produced by epithelial cells of lining the respiratory, digestive, and reproductive tracts, we investigated whether SLPI in BALF is also influenced by inhalation of high concentration of SO$_2$. Interestingly, SLPI in BALF was also elevated in SO$_2$ group. SLPI has antiprotease activity and plays an important role in neutralizing enzymes such as neutrophil elastase to prevent excessive tissue damage during inflammation. For this reason, we investigated if the cathepsin K activity of BALF can be inhibited by SLPI. The result showed that the cathepsin K activity in BALF of SO$_2$ group was significantly lower than that of control group. However, the serum cathepsin K activity in SO$_2$ group was not influenced, which suggested that the inhibitory effect of SLPI to cathepsin K activity was limited to the airway and lung, instead of the whole body. It was well documented that SLPI could inhibit cathepsin G activity, but our result indirectly showed that SLPI could also inhibit cathepsin K activity. As for the mechanism underlying that SLPI expression and secretion were upregulated by inhalation of high concentration of SO$_2$, we speculated that there were two possibilities. One was that SO$_2$ acted directly on the epithelial cells of respiratory tract and induced the expression and secretion of SLPI. Another was that SO$_2$ caused inflammation in airway and lung, and the expression and secretion of SLPI were induced by the inflammation. It was reported that the expression of SLPI could be increased by proinflammatory stimuli such as TNF-α and IL-1β. This was supported by the histopathological results in this study, which showed the lymphocytes infiltration in the lung of SO$_2$ group. The further studies are needed to elucidate the detail mechanism.

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**Conflicts of interest**

There are no conflicts of interest.

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