Histol Histopathol (2014) 29: 1107-1111
DOI: 10.14670/HH-29.1107

http://www.hh.um.es

Histology and Histopathology
Cellular and Molecular Biology

Review

Effect of endogenous sulfur dioxide in regulating cardiovascular oxidative stress

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Summary. In the middle of the 1980s, nitric oxide received extensive attention because of its significant effects in life science. Then, carbon monoxide and hydrogen sulfide were discovered to be gasotransmitters playing important roles in regulating cellular homeostasis. As a common air pollutant, sulfur dioxide (SO_2) can cause great harm to the human body by producing free radicals, which causes oxidative damage to various organs. Recently, endogenous SO_2 was found to be produced in the cardiovascular system and might be a bioactive molecule regulating the physiological activities including cardiovascular oxidative stress.

Key words: Sulfur dioxide, Cardiovascular, Oxidative stress

Introduction

Sulfur dioxide (SO_2) is one of the most common pollutants in the atmosphere and is considered greatly harmful to body. However, research has shown that in mammals, SO_2 can be produced by the metabolism of sulfur-containing amino acids (Ji et al., 1995). Recently, this pathway of endogenous SO_2 generation was found in the cardiovascular system (Du et al., 2008). There, SO_2 plays an important role in physiological functions such as vascular dilation (Du et al., 2006, 2008), inhibition of cardiac functions (Zhang et al., 2008, 2009), and improvement of vascular remodeling (Jin et al., 2008; Zhao et al., 2008; Sun et al., 2010). It is also involved in pathophysiological processes of cardiovascular diseases such as pulmonary arterial hypertension, systemic hypertension (Jin et al., 2008; Sun et al., 2010), myocardial ischemia-reperfusion injury (Zhang et al., 2008, 2009; Wang et al., 2011) and atherosclerosis (Li et al., 2011). Thus, endogenous SO_2 might be a novel gasotransmitter in the regulation of cardiovascular functions (Wang et al., 2010). Numerous studies have shown that oxidative stress is one of the most important pathological processes of many cardiovascular diseases, and oxygen free radicals are involved in various pathophysiological processes of the cardiovascular system, such as atherosclerosis and hypertension.

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General properties of SO_2

SO_2 is the most common sulfur oxide and is mainly generated from coal and oil combustion. It is a colorless toxic gas with a characteristic odour at room temperature. Its density is greater than air, which makes it easy to liquefy (at -10°C) and dissolve in water. SO_2 is believed to be the main cause of respiratory damage and is easily absorbed by the moist mucous surface to generate sulfurous acid and sulfuric acid, which have a strong irritating effect on the respiratory mucosa. Also, excessive inhalation of SO_2 can cause pulmonary edema (Koshino et al., 1990). The combined effects of SO_2 and dust lead to alveolar wall fibrosis and ultimately, emphysema. The toxic effects of SO_2 involve three components; systemic toxic effects, chromosome

breaking agents and weak mutagenic effects (Meng and Zhang, 1999; Meng et al., 2004), which lead to the oxidative stress damage seen in liver, lung, kidney, heart, spleen, brain and other organs (Meng, 2003).

A sulfur atom in SO_2 is tetravalent, so SO_2 can be both oxidized and reduced. SO_2 is oxidized to sulfuric acid mist and sulfate aerosols in the atmosphere, which are important precursors of environmental acidification. Following entrance into the body, SO_2 combines rapidly with water, producing sulfuric acid in the respiratory tract, which is decomposed to derivatives including sulfite and bisulfite (Shapiro, 1977) when dissolving in body fluids. SO_2 derivatives can then exert their toxic effects on different tissues in the body by circulating in the blood stream.

Generation and metabolism of endogenous SO_2

In vivo, the metabolism of sulfur-containing amino acids can produce L-cysteine, which can continue to generate SO_2. The process is as follows: L-cysteine is oxidized to L-cysteinesulfinate by cysteine dioxygenase (CDO), and the latter produces taurine or pyruvate through two metabolic pathways, one that decarboxylates L-cysteinesulfinate and one involving the action of an aminotransferase (Stipanuk, 1986; Sun et al., 2010) (Fig. 1). The pathway of decarboxylation allows L-cysteinesulfinate to generate CO_2 and hypotaurine through cysteinesulfinate decarboxylase (CSD), and hypotaurine can be further oxidized to taurine. The other pathway is the generation of β-sulfinylpyruvate from L-cysteinesulfinate through aspartate aminotransferase (AAT). β-sulfinylpyruvate is then spontaneously broken down to pyruvate and SO_2. In the body, SO_2 combines with water to produce SO_3^{2-}, and the latter generates SO_4^{2-} through oxidation by sulfite oxidase and is finally excreted in urine through the kidneys. The two metabolic pathways of L-cysteinesulfinate described above are active, and the degree of activity depends on the relative activity of aminotransferase and decarboxylase.

SO_2 can be generated by intracellular H_2S through other pathways. First, H_2S is oxidized to thiosulfate under the effect of heme compounds, metal–protein complexes and ferritin. Thiosulfate then reacts with glutathione (GSH) to form sulfite or SO_2 through catalysation of thiosulfate reductase (Shapiro, 1977; Kamoun, 2004). Within the activated neutrophile granulocyte, H_2S can be oxidized to form sulfite or SO_2 through NADPH oxidase (Mitsuhashi et al., 2005).

A key enzyme for generating endogenous SO_2 is AAT, consisting of two kinds of isozymes, AAT1 and AAT2, which are mainly distributed in the cytoplasm and cellular mitochondria, respectively (DeLorenzo and Ruddle, 1970). In 2008, Du et al. found that SO_2 was present in arteries, with the highest content in the aorta (5.55±0.35 μmol/g. protein). However, the content of AAT was relatively lower in aorta than renal arteries (88±11 vs. 188±30 U/g. protein). The expression of AAT1 and AAT2 does not differ in blood vessels and both are expressed in vascular endothelial and vascular smooth muscle cells (Du et al., 2008). Meng et al. (2009) used high-performance liquid chromatography and measured the mean concentration of endogenous SO_2 at (127.76±31.34) and (16.77±8.24) μmol/L in vascular tissue and plasma, respectively, in normal rats, for higher content in vascular tissue than plasma. Also, endogenous SO_2 was present in rat aortic endothelial and vascular smooth muscle cells cultured *in vitro*, with the content higher in the former than latter cells (Meng et al., 2009). Luo et al. (2011) studied SO_2 production and distribution of AAT and CDO in different rat tissues and found the highest content of SO_2 in the stomach, followed by the right ventricle and left ventricle. However, the liver content of SO_2 was low. AAT activity and mRNA expression of AAT1 were highest in the left ventricle, but the highest AAT1 protein expression was in the right ventricle. The mRNA levels of AAT2 and CDO were highest in the liver, but the protein content of AAT2 was highest in the renal medulla, and that of CDO was highest in liver. In addition, AAT1and AAT2 were

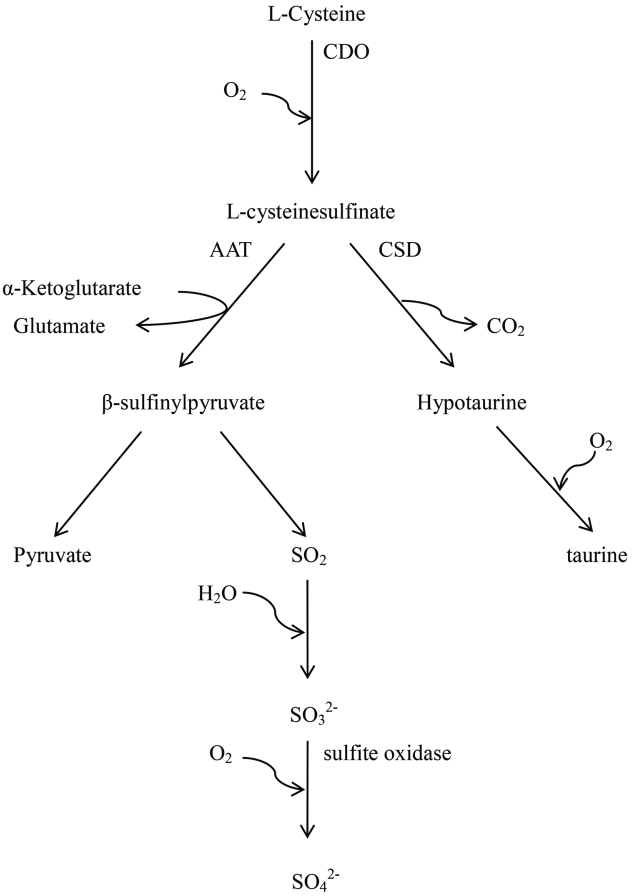

Fig. 1. Pathway of endogenous production of SO_2, with involvement of conversion of L-cysteine to SO_2. CDO: cysteine dioxygenase, AAT: aspartate aminotransferase, CSD: cysteinesulfinate decarboxylase.

mainly distributed in the cytoplasm in all tissues (Luo et al., 2011).

Balazy et al. found that acetylcholine (Ach) stimulated SO_2 production in coronary artery rings of pigs *in vitro* (Balazy et al., 2003). Meng et al. studied the regulation of Ach and norepinephrine (NE) in terms of generation of endogenous SO_2 in rats (*in vitro*-cultured vascular endothelial cells, smooth muscle cells and *in vivo* thoracic aorta vascular tissue), finding that Ach could dose-dependently stimulate the generation of endogenous SO_2 in cultured vascular endothelial cells, smooth muscle cells, and the thoracic aorta. In contrast, NE inhibited the generation of endogenous SO_2. The generation of endogenous SO_2 could be regulated by certain organic substances, such as Ach and NE, which supports that SO_2 may be an endogenous active substance in vascular tissues.

SO_2 and cardiovascular oxidative stress regulation

During oxidative stress, the body experiences various harmful stimulations, with overproduction of active molecules such as reactive oxygen species (ROS) and reactive nitrogen species (RNS), thus exceeding the capacity of the anti-oxidative system and leading to imbalanced oxidative and anti-oxidative systems, which results in tissue damage. ROS include superoxide anions ($.O_2^-$), hydroxyl free radicals (.OH), hydrogen peroxide (H_2O_2), RNS nitric oxide (.NO), nitrogen oxide ($.NO_2$), and nitrite peroxide ($.ONOO^-$).

The organism antioxidant system consists of antioxidant enzymes and antioxidants, including superoxide dismutase (SOD), GSH peroxidase (GSH-Px), catalase (CAT) and reduced GSH. GSH, synthesized from the amino acids L-cysteine, L-glutamic acid and glycine, plays an important role in scavenging free radicals and protecting against oxidative stress and is the most important intracellular antioxidant. GSH is oxidized to oxidized glutathione (GSSG) by GSH-Px under the action of the oxidants. Consequently, when oxidative stress occurs, the GSH/GSSG ratio decreases, by which we can estimate peroxidation damage. SOD is the superoxide radical scavenging factor naturally occurring within the organism, converting harmful superoxide radicals to H_2O_2, which is broken down immediately into harmless water by GSH-Px and CAT. In this way the three enzymes form a complete antioxidant chain. The pentose phosphate pathway is a pathway of glucose metabolism in the body that can produce a large number of reduced NADPH, maintaining GSH in the reduced form. Glucose-6-phosphate dehydrogenase (G6PD) is the key enzyme of the pentose phosphate pathway.

SO_2 antagonizes oxidative stress injury in atherosclerosis in rats

Atherosclerosis (AS) is a complex disease with multiple factors, and its mechanism is not yet fully clarified. AS is related to increased levels of blood lipids, especially cholesterol, which become incorporated in arterial walls and gather in local depositions, thus forming atherosclerotic plaques with foam cells, necrosis, fibrosis and inflammation. Low-density lipoprotein becoming oxidized low-density lipoprotein by ROS is a central part of AS lesions (Mitra et al., 2011).

In studying the regulation of SO_2 in AS lesions, Li et al. found that the SO_2 level and AAT activity were reduced in plasma and arteries of atherosclerotic rats. Also, GSH-Px and SOD activity was decreased, whereas MDA content was increased in plasma, and SOD1 and SOD2 protein expression was decreased in artery tissue. On treatment with an SO_2 donor (Na_2SO_3/$NaHSO_3$, 0.54 $mmolkg^{-1}$: 0.18 $mmolkg^{-1}$), GSH-Px and SOD activity was increased, but MDA content decreased in plasma; SOD1 and SOD2 protein expression was increased in arterial tissue and AS lesions were significantly reduced. Thus, AS lesions might be linked to a downregulated SO_2/AAT pathway because SO_2 successfully prevented the progression of atherosclerosis by inhibiting lipid peroxidation (Li et al., 2011).

SO_2 increases anti-oxidative ability in rats with pulmonary hypertension induced by monocrotaline

Pulmonary hypertension (PH) is a pathological syndrome with increased pulmonary resistance caused by increased pressure in the pulmonary circulation, eventually leading to right-sided heart failure. Pulmonary vascular remodeling is the essential pathological feature of PH, with vascular smooth muscle cell proliferation and hypertrophy, endothelial cell dysfunction, and extracellular matrix deposition as the main mechanisms. Excessive accumulation of ROS causes oxidative stress in tissues and cells, which directly results in oxidative damage and/or activating redox signaling pathways, thus promoting pulmonary vascular remodeling (DeMarco et al., 2008). Monocrotaline (MCT) is a cytotoxic compound extracted from the seeds of purple lily. In adult rats, MCT can cause pulmonary artery remodeling and lead to pulmonary hypertension and right ventricular hypertrophy (Lappin and Roth, 1997). Oxidative stress promotes MCT-induced PH (Aziz et al., 1997; Kamezaki et al., 2008).

In the past, most studies have mainly focused on the role of SO_2 in oxidative damage of tissues. Jin et al. first explored the significance of endogenous SO_2 in regulating MCT-induced PH (Jin et al., 2008). SO_2 content in MCT-induced PH rat lung tissue as well as AAT enzyme activity of plasma and lung tissue were increased. However, the administration of AAT enzyme inhibitors increased the ratio of mean pulmonary artery pressure and weight of the right ventricle (left ventricle plus septum), and damaged muscularized pulmonary artery microstructure, which suggests that increased endogenous SO_2 in MCT-induced PH might play a

protective role. SO_2 may increase the anti-oxidative capacity in rats with MCT-induced PH. Also, administration of an SO_2 donor (Na_2SO_3/$NaHSO_3$, 72.3 mg/kg) significantly increased lung-tissue GSH-Px and SOD activity as well as plasma GSH-Px and CAT activity, so SO_2 may improve the antioxidant ability of rats.

SO_2 antagonizes myocardial oxidative stress injury induced by isoproterenol

Myocardial injury is a common pathological feature of many heart diseases. The mechanisms involve hypoxia, calcium overload and oxidative stress. Isoproterenol (ISO) has toxic effects on the heart by producing oxygen free radicals, which then interact with thiol (Singal et al., 1981). These oxygen free radicals can cause cardiomyocyte membrane lipid peroxidation, membrane structure damage, and damage to various different proteins and enzymes in the cell, thus leading to myocardial damage (Noronha-Dutra et al., 1988).

In researching the endogenous pathway of SO_2 in myocardial oxidative stress regulation, Liang et al. found that ISO-treated rats showed damaged cardiac structure, reduced heart function, and downregulated endogenous SO_2/AAT pathway. At the same time, myocardial tissue production of oxidative products and H_2O_2 was increased, but the production of GSH-Px and SOD was significantly reduced. In other words, myocardial oxidative stress was increased with decreased antioxidant capacity. However, administration of an SO_2 donor (Na_2SO_3/$NaHSO_3$, 85 mg/kg) to ISO-treated rats was able to significantly improve myocardial and mitochondrial structure, and the ISO-induced myocardial injury was significantly improved with improved cardiac function. At the same time, myocardial tissue GSH and SOD activities were increased, with reduced H_2O_2 content, so myocardial oxidative stress products were reduced because of increased cardiac antioxidant capacity. However, administration of SO_2 alone decreased the levels of myocardial GSH-Px and SOD activity, with no change in oxygen free radicals, which illustrates the potentially negative effects of SO_2 administered alone in myocardial tissue. These results suggest that ISO-induced myocardial injury might be associated with a downregulated SO_2/AAT pathway and that SO_2 can improve ISO-induced myocardial injury and heart function by increasing the antioxidant capacity of the myocardium (Liang et al., 2011).

Conclusions

Inhalation of high concentrations of SO_2 can damage the GSH redox system, and the activity of SOD, GSH-Px, GSH, GSH/GSSG, GST and G6PD is decreased in rats exposed to SO_2, along with increased ROS concentration, which leads to oxidative damage of various tissues and organs (Geng and Meng, 2003; Meng, 2003; Wu and Meng, 2003). However, in recent years, we have found that cardiovascular tissue can generate relatively low levels of endogenous SO_2, which seems to play an important role in antagonizing oxidative stress injury and alleviating cardiovascular damage. SO_2 has been found to have important pathophysiologic significance in terms of regulating pulmonary hypertension, myocardial ischemia-reperfusion injury, and atherosclerosis. In conclusion, this novel gasotransmitter can be added to the family of previously discovered endogenous gasotransmitters nitric oxide, carbon monoxide and hydrogen sulfide, and seems to play an important part in the cardiovascular system, where it balances and regulates oxidative and anti-oxidative pathways. Studying the role of SO_2 in cardiovascular oxidative stress regulation can deepen our understanding of the pathogenesis of different cardiovascular diseases and help in developing new methods to prevent and control these diseases. However, the molecular mechanisms by which endogenous SO_2 regulates cardiovascular oxidative stress still need further investigation.

Acknowledgements. Supported by National Natural Science Foundation of China (31130030), Major State Basic Research Development Program (2012CB517806 and 2011CB503P04)

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Accepted April 9, 2014