Sulfur Dioxide: a Novel Gaseous Signal in the Regulation of Cardiovascular Functions

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Abstract: The atmospheric pollutant sulfur dioxide (SO_2) is endogenously generated from the normal metabolism of sulfur-containing amino acids through the aspartate aminotransferase pathway. SO_2 is produced in cardiovascular tissues, and the aspartate aminotransferase mRNA is localized in endothelia and in vascular smooth muscle cells near the endothelial layer. Recent studies explored the physiological and pathophysiological effects of endogenous SO_2 on the cardiovascular system, and various potential mechanisms were found. These discoveries suggest a novel role of endogenous SO_2 in the modulation of the cardiovascular system and provide a basis for new treatments for cardiovascular diseases.

Keywords: Cardiovascular, gaseous signal, sulfur dioxide, pathophysiology, physiology, toxicology.

INTRODUCTION

Sulfur dioxide (SO_2) is a colourless dense gas that smells pungent. It enters the atmosphere primarily from anthropogenic activities, such as the combustion of coal and residual fuel oil. Hydrogen sulfide (H_2S), derived from the eruptions of volcanoes and the biological decay of organic matter, is rapidly oxidized to SO_2 when entering the atmosphere. The reduction of sulfate is another source of sulfur in nature [1]. The biogeochemical sulfur cycle comprises all of the sulfur compounds. SO_2 is a major member of this cycle and plays a critical role in maintaining the balance of environmental sulfur [2]. As a well-known pollutant gas, SO_2 is involved in the formation of photochemical smog and acid rain, both of which are harmful to humans, animals and plants [1]. However, SO_2 is widely used in the manufacture of food, wine and cosmetics as an antimicrobial agent or antioxidant [2].

Balazy *et al.* speculated that SO_2 might be a type of endothelium-derived hyperpolarizing factor (EDHF) because it shares several features with EDHFs [3]. As well, SO_2 is generated endogenously from sulfur-containing amino acids (SAAs) [4]. Researchers have therefore focused on the physiological functions of SO_2 other than the only environmental influences or industrial uses, particularly the regulatory effects on the cardiovascular system.

The aim of this review is to (i) describe the endogenous generation and metabolism of SO_2, (ii) briefly summarize the toxicological effects of SO_2, and (iii) give an overview of the physiological and pathophysiological significance of endogenous SO_2 in the cardiovascular system.

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ENDOGENOUS GENERATION AND METABOLISM OF SO_2

SO_2 can be produced from the normal metabolism of SAAs [5]. Five types of SAAs are present in the human body: methionine (Met), cysteine (Cys), cystine, homocysteine and taurine. In mammals, Met is an essential amino acid, whereas Cys is considered a semi-essential amino acid because it can be converted from homocysteine and serine *via* trans-sulfuration, and its sulfur stems from Met sulfur indirectly [5, 6]. Cys can also be supplied in the diet [5].

L-Cys is a vital precursor for the endogenous synthesis of SO_2 [5,7]. *L*-Cys is oxidized to *L*-cysteinesulfinate catalyzed by cysteine dioxygenase (CDO) and then *L*-cysteinesulfinate is transaminated to form β-sulfinylpyruvate catalyzed by aspartate aminotransferase (AAT) [5,7]. The metabolite β-sulfinylpyruvate decomposes spontaneously to pyruvate and SO_2 [7]. Some of the SO_2 is hydrated to sulfite, which is subsequently oxidized by sulfite oxidase to sulfate, and the latter is excreted into the urine [8]; but some SO_2 can exist in the gaseous form, thus evading this reaction [3]. Another pathway for *L*-cysteinesulfinate metabolism involves *L*-cysteinesulfinate being decarboxylated by cysteinesulfinate decarboxylase (CSD) to carbon dioxide (CO_2) and hypotaurine. Most of the latter is subsequently oxidized to taurine [5,7]. The 2 pathways compete with each other [7]. Griffith, by researching the inhibitor of CSD, found that the activities of AAT and CSD determined the reaction tendency of *L*-cysteinesulfinate [9]. Some sulfate is activated to form 3'-phosphoadenosine-5'-phosphosulfate (PAPS), which serves as a sulfate donor endogenously [5]. H_2S, metabolized from SAAs catalyzed by cystathionine β-synthase (CBS) or cystathionine γ-lyase (CSE) [10], can also be converted into SO_2 derivatives, such as thiosulfate, sulfite and sulfate, through a series of oxidations [5,11]. Moreover, *L*-Cys is a source of endogenous SO_2 as well as a precursor for glutathione (GSH) [12]. Some enzymes in the network of *L*-Cys metabolism

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play an essential role in the generation of sulfate, taurine and GSH in liver, including AAT, CDO, CSD and γ-glutamylcysteine synthetase (GCS, a key enzyme catalyzing *L*-Cys to GSH) [12]. The metabolism of *L*-Cys reveals a potential relationship between endogenous SO_2 and cellular redox status. For example, some researchers found overdosed *L*-Cys could disturb the redox status in the human body [13] (Fig. **1**).

Mitsuhashi *et al*. reported that activated neutrophils obtained from the inflamed peritoneal cavity spontaneously yielded sulfite in higher amounts than non-stimulated neutrophils isolated from the blood [14]. They further explained that sulfite production depended on the availability of PAPS [14]. Using an *in vitro* model of rat neutrophil activation induced by lipopolysaccharide, Mitsuhashi *et al*. demonstrated that oxidative stress by activated neutrophils could convert H_2S to sulfite with the assistance of reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase; in activated neutrophils, 15% H_2S was converted to sulfite within 30 min [15] (Fig. **1**).

Balazy *et al.* analyzed the headspace gas of incubation of porcine coronary arterial rings and cardiac muscle tissues in Krebs buffer [3]. They used gas chromatography–mass spectrometry to detect 2 gases: carbonyl sulfide and SO_2. SO_2 formation was observed in the porcine coronary artery after incubation with calcium ionophore [3]. SO_2 may be produced by an intracellular thiol, and Balazy *et al.* also speculated that SO_2 could originate from the disproportion or oxidation of the very unstable molecule sulfur monoxide [3].

Humphries *et al.* confirmed that bovine aortic endothelial cells and smooth muscle cells could use Cys as a sulfate source [16]. On the basis of the abovementioned conclusions, Du *et al*. first measured endogenous SO_2 pathway in cardiovascular system, including SO_2 concentration in plasma and different tissues of Wistar rats and evaluated the distribution of AAT and its mRNA [17]. SO_2 concentration in rat plasma was 15.54±1.68 μmol/L. Aortic tissue had the highest content of SO_2, up to 5.55±0.35 μmol/g protein, followed by (in μmol/g protein) pulmonary arteries (3.27±0.21), mesenteric arteries (2.67±0.17), tail arteries (2.50±0.20), and renal arteries (2.23±0.19) [17]. The activity of AAT of the renal arteries was higher than that in any of the other tissues they inspected [17]. Du *et al*. also assessed AAT mRNA expression in endothelial cells and vascular smooth muscle cells beneath the endothelial layer [17]. However, in 1977, because of the limitations of methodology, sulfite in normal human plasma and urine were not detectable, unless in cases of sulfur oxidase deficiency [18]. Later, Allena *et al*. gave the reference range for total serum sulfite: 0~9.85 μmol/L in normal human beings by high-performance liquid chromatography with fluorescence detection [19]. In patients with acute pneumonia, Mitsuhashi *et al.* found the serum sulfite increased to 3.75±0.88 μmol/L as compared with control subjects (1.23±0.48 μmol/L) [20]. The authors also investigated serum level of sulfite in Wistar rats: the normal level was 1.3±0.23 μmol/L [15]. Recently, Meng *et al.* demonstrated that the basal sulfite level of plasma in Wistar rat was 12.59±9.03 μmol/L [21]. The latest research showed that endogenous SO_2 was mainly generated in endothelial cells of vascular tissues [22] in accordance with previous findings [17].

TOXICOLOGICAL EFFECTS OF SO_2

As a gaseous atmospheric toxin, SO_2 has been linked to various symptoms (e.g., cough and dyspnea) [23]. For example, SO_2 could produce pulmonary neutrophillic inflammation and aggravate cough [24]. Much research has suggested that SO_2 exposure could decrease heart rate variability and be related to cardiac dysrhythmia, which might be a potential mechanism responsible for cardiovascular disease morbidity and mortality [25-28]. In pre-existing asthma subjects, inhalation of SO_2 could exacerbate this disease, possibly by inducing asthma-related gene expression or regulating apoptosis-related genes in the lung [29-31]. In spontaneously hypertensive rats (SHRs), airway inflammation tended to be induced by SO_2 [32]. Epidemiological research involving more than 4 million visits to hospital emergency departments showed that exposure to ambient air pollution increased the susceptibility to adverse cardiovascular events among people with pre-existing cardiovascular and respiratory illnesses [33]. According to these investigations, subjects with pre-existing cardiopulmonary disease had greater SO_2 sensitivity in general. As well, large-artery endothelial functions could be impaired by exposure to air pollution (especially SO_2 and nitric oxide [NO]) in healthy young male volunteers [34].

Inhaling SO_2 can cause systemic oxidative damage [35]. Inhalation of SO_2 was found to lead to oxidative injury of cardiovascular, respiratory, digestive, reproductive, endocrine and nervous systems [36-41]. SO_2 inhaled by humans or mammals is readily hydrated to sulfurous acid, which subsequently dissociates to yield sulfite ions and bisulfite ions. The 2 ions exist in blood circulation, and sulfite ions can be oxidized to sulfate ions, which is, potentially, an essential process of modulating the level of sulfite *in vivo* [42]. SO_2 toxicity is sometimes present with respect to sulfite ions. The formation of sulfur- and oxygen-centered free radicals such as $SO_3^{\cdot-}$, $SO_4^{\cdot-}$and $SO_5^{\cdot-}$ contributes to SO_2-induced toxicity during the oxidation of SO_2 derivatives [43]. As well, the change in the redox status of myocytes induced by SO_2 might be the etiologic cause of myocardial injury [44].

DNA damage caused by SO_2 and sulfite ions includes triggering an increase in the frequencies of chromosomal aberrations, sister chromatid exchanges, micronuclei formation and DNA–protein crosslinking in mammalian cells [36,45,46].

Furthermore, SO_2 could, alone or with other toxic agents, upregulate the expression of oncogenes (c-myc, H-ras, c-fos, c-jun and Ki-ras) and downregulate that of tumor suppressor genes (p53, p16 and Rb) in lung, liver and bronchial epithelial cells [47-50].

Zhang *et al.* proposed that glutamate dehydrogenase (GDH) could be inhibited by sulfite ions directly, which could give rise to sulfite oxidase deficiency in human infants [51]. The defect of this enzyme, sulfite oxidase, is attributed to neurologic abnormalities [18]. The inhibitory effect of a micromolar concentration of sulfite on mitochondrial GDH could induce a prompt increase in reactive oxygen species in Madin–Darby canine kidney cells, associated with depletion of intracellular adenosine triphosphate (ATP) [52].

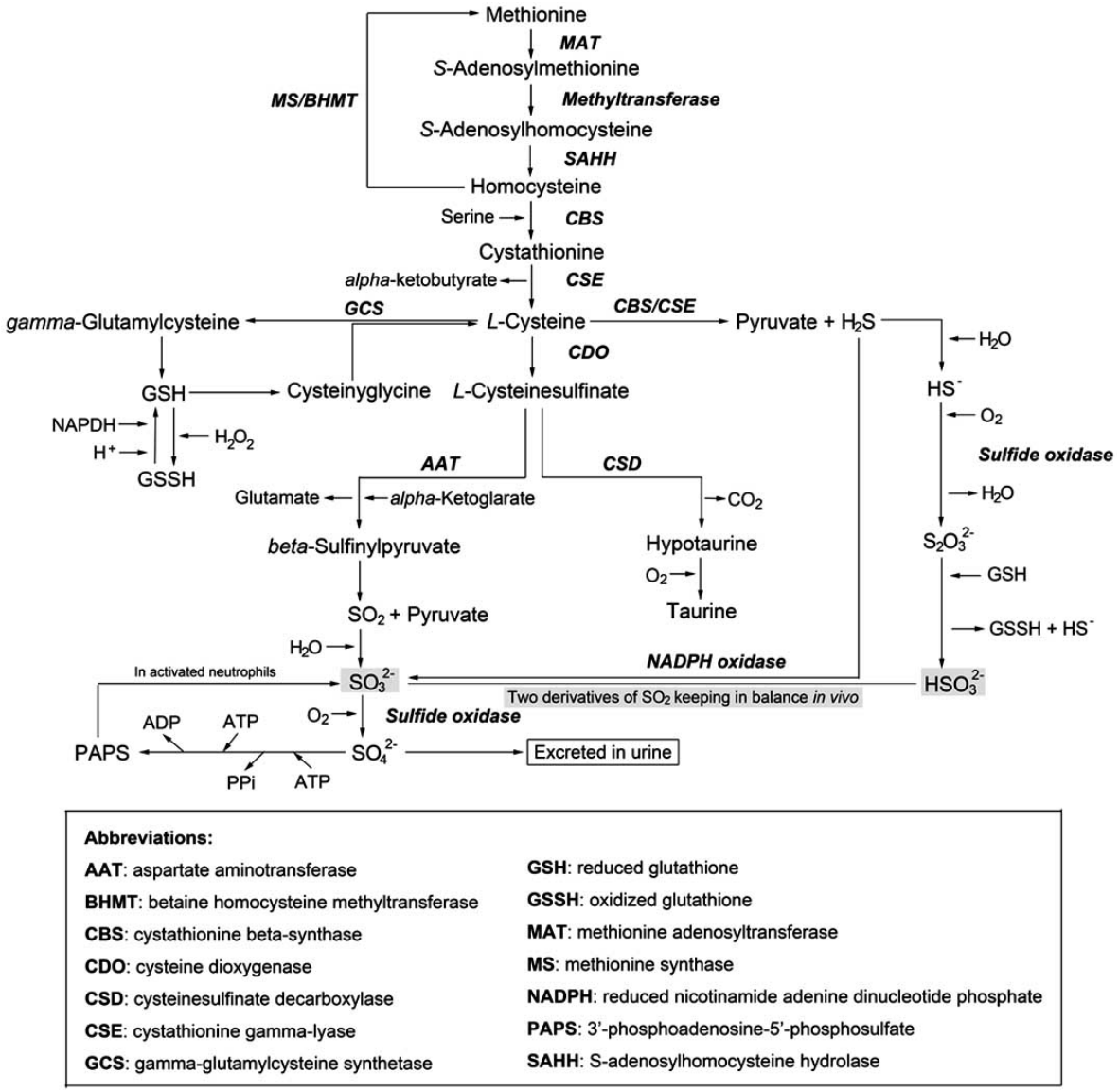

Fig. (1). Endogenous generation and metabolism of sulfur dioxide.

PHYSIOLOGICAL FUNCTIONS OF ENDOGENOUS SO_2 IN THE CARDIOVASCULAR SYSTEM

Meng *et al.* reported that short-term exposure to SO_2 (10–40 ppm) caused both a dose- and time-dependent reduction of blood pressure in Wistar rats [53]. The authors concluded that SO_2 is a systemic toxic agent to the cardiovascular system [53]. However, the physiological effects of endogenous SO_2 on the cardiovascular system have only been revealed recently.

Du *et al.* first found that with incubation with SO_2 derivatives (mixture of sodium bisulfite and sodium sulfite, 1:3 M/M in neutral solution), the isolated aortic rings of rats exhibited greater relaxant reactivity in a dose-dependent manner, but rings incubated with *L*-aspartate-*β*-hydroxamate (HDX), the inhibitor of AAT, which suppressed the production of endogenous SO_2, showed stronger vasoconstriction than that of the control group [54]. Moreover, this physiological vasorelaxant regulatory mechanism may be connected with the calcium (Ca^{2+}) channel and ATP-sensitive potassium (K_{ATP}) channel [54]. Later, the authors determined that the endogenous SO_2 and its derivatives at a low concentration (25–100 μmol/L) had a vasorelaxant effect *via* the SO_2/AAT pathway, and the potential mechanism was not endothelium dependent but was related to the L-type Ca^{2+} channel [17]. Meng *et al.* reported that the vasodilating function of SO_2 was independent of the synthesis of NO and the intact vascular endothelium but partially related to the signal transduction pathway of prostacyclin (PGI_2)–adenylate cyclases (AC)–cAMP–protein kinase-A [55,56]. Recently, the vasorelaxant effect of SO_2 at basal and low concentrations was found to be endothelium dependent and accompanied by activation of NO synthase (NOS), while at a high concentration was endothelium independent and relied on the inhibition of the voltage-gated Ca^{2+} channel with the opening of the K_{ATP} and Ca^{2+}-activated potassium (K^+) channel [57,58]. The activation of NOS involved in this vasorelaxant mechanism contributing to the potential interaction of NO and SO_2 and the synergy of NO and endogenous SO_2 in vasoactivity has been shown [21]. In addition, SO_2 level in vascular tissue

could be increased by acetylcholine and decreased by noradrenaline [22], which further clarified the association of endogenous SO_2 and vasoactivity.

SO_2 derivatives could give rise to myocardial electrophysiological changes by inducing K^+, Ca^{2+} and sodium (Na^+) currents and modulating voltage-gated K^+ channels, L-type Ca^{2+} channels and voltage-gated Na^+ channels [59-61]. The channels shared the similar underlying mechanism, with possible involvement of the switch to the redox status of amino acid residues of these channel protein subunits [59-61]. SO_2 could also inhibit both inward and outward Na^+-Ca^{2+} exchange currents in myocytes, thus leading to an increase in myocardial intracellular free Ca^{2+} concentration [62].

PATHOPHYSIOLOGICAL EFFECTS OF ENDOGENOUS SO_2 ON THE CARDIOVASCULAR SYSTEM

Little is known about the pathophysiological significance of endogenous SO_2 in cardiovascular diseases other than the toxicological outcomes of exogenous SO_2.

Zhao *et al.* first reported that the systemic blood pressure of 5-week SHRs was higher than that of Wistar Kyoto rats, whereas the plasma SO_2 content of SHRs was decreased by 44% as compared with the controls [63]. With SO_2 derivatives treatment, the systolic blood pressure of SHRs decreased significantly as compared with the untreated group. As well, the ratio of media to lumen radius, media pressure and the proliferative index of smooth muscle cells in the aorta of SHRs treated with SO_2 derivatives were lower than those of untreated rats [64, 65]. Thus, the decreased endogenous SO_2 production might be involved in the process of hypertension.

Pulmonary hypertension (PH) is characterized by an elevation in mean pulmonary arterial pressure and pulmonary vascular remodeling. The latter remodeling consists of hypertrophy and hyperplasia of various cells, together with accumulation of the extracellular matrix [66]. Sun *et al.* found that rats under hypoxic conditions exhibited significantly decreased SO_2 levels in plasma and lung tissue, with increased pulmonary artery pressure, pulmonary vascular remodeling and vascular inflammation [67]. This finding implied that the development of hypoxic PH was accompanied by the downregulation of endogenous SO_2 concentration. Tian *et al.* discovered that SO_2 derivatives could significantly lower mean pulmonary arterial pressure and systolic pulmonary arterial pressure of hypoxic pulmonary hypertensive rats, with no obvious effect on diastolic pulmonary arterial pressure [68]. As well, SO_2 and its derivatives could alleviate pulmonary vascular remodeling in hypoxic PH by inhibiting collagen type I and III agglomeration in vascular walls [69] and by inhibiting vascular smooth muscle cell proliferation, possibly by inactivating the extracellular signal-regulated kinase/mitogen-activated protein kinase signaling pathway [67]. Inflammation is involved in hypoxic PH [70]. Apart from preventing pulmonary vascular remodeling, SO_2 could downregulate the expression of the 2 vital inflammation factors, nuclear factor-kappa B (NF-κB) and intercellular adhesion molecule-1 (ICAM-1) [71]. This result suggested the protective part SO_2 plays in regulating inflammation in experimental hypoxic PH [71].

SO_2 inhalation was found to decrease activities of superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) in several organs, weaken catalase (CAT) activities in liver and reduce GSH content, which suggests that SO_2 causes systemic oxidative damage [35]. However, Jin *et al.* discovered in a monocrotaline (MCT)-treated rat model that SO_2 derivatives could improve the activities of antioxidative enzymes, including SOD, GSH-Px and CAT, whereas the activities of these enzymes were decreased when endogenous SO_2 was inhibited by HDX [72]. This finding indicated that endogenous SO_2 could enhance the antioxidative effect in MCT-induced oxidative stress [72]. The authors also found that SO_2 alleviated structural remodeling in the small pulmonary artery and improved hemodynamic status in MCT-induced PH [72]. They concluded that the SO_2/AAT pathway may participate the protection process in MCT-induced PH [72].

The formation of oxygen-derivated free radicals is responsible for the cytotoxic process during ischemia. Reperfusion of the myocardium in several aspects and the peroxidation of lipid components of cellular and mitochondrial membranes is the main cellular damage during myocardial infarction [73]. Zhang *et al.* evaluated the role of SO_2 in isolated rat heart models of ischemia and reperfusion [74]. During reperfusion, inhibiting the generation of endogenous SO_2 reduced the permeability of the myocardial cell membrane and the production of myocardial malondialdehyde, a product of lipid peroxidation, and elevated the GSH content of myocardium [74]. This discovery implied that myocardial injury *via* SO_2 may be associated with increased lipid peroxidation and decreased GSH in the myocardium [74].

CONCLUSIONS AND PERSPECTIVES

In the 1980s, the era of "gas biology" commenced with the profound understanding of NO, but NO is not the only biologically active gas in the human body. Carbon monoxide (CO) and, more recently, H_2S, have been shown to have similar significance [75, 76]. The concept of the "gaseous signal family" was put forward. The 3 members carry out their functions individually and in a "cross-talk" manner [75,76]. Whether SO_2 should be recognized as a gaseous transmitter depends more on its physiological and pathophysiological effects in regulating the cardiovascular system than its toxicological influences on human health with abnormal atmospheric concentrations. SO_2 and H_2S share the same endogenous source, namely SAAs [5,11], and the 2 gases can transform into each other (or their derivatives) *via* several biochemical reactions [5,11,15]. We suggest that the relationship between SO_2 and H_2S may be a key factor linking SO_2 and gaseous signals. As well, the synergistic vasorelexant function of SO_2 and NO is another path to combine SO_2 with gaseous signal family network.

Studies of SO_2 are not as simple as those of H_2S because AAT is probably not a specific enzyme catalyzing only the reaction of SO_2 generation. AAT has a major role in various metabolisms (e.g., aspartate biosynthesis *via* transamination of oxaloacetate by glutamate) and is also indirectly connected to the urea cycle and tricarboxylic acid cycle [77]. Multiple factors should therefore be taken into consideration when its inhibitor HDX is used in research. However, this

complication of SO_2 existing in organisms indicates that its function *in vivo* results from its chemical and physical properties as a gaseous molecule and from its network-like interaction with the body energy, protein and inorganic salts metabolism. A tiny alteration in its level might give rise to a "butterfly effect" in the body function.

Drugs designed according to the biological activity of NO have revolutionized the therapy of cardiovascular diseases. Many functions of H_2S have been revealed in the cardiovascular system [78-80], and a new water-soluble H_2S-releasing molecule, GYY4137, was recently reported to have potential clinical significance in the cardiovascular system [81]. Although more unknowns about SO_2 are waiting to be elucidated in further explorations, the results obtained thus far suggest the novel role of SO_2 in modulating the cardiovascular system and provide a basis for new treatments for cardiovascular diseases.

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