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Review

Potential therapeutic effect of SO_2 on fibrosis

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Summary. Fibrosis is a pathological feature of most chronic diseases and leads to the dysfunction of various organs. However, there is currently no effective method for treating fibrosis. In recent years, a small gas, sulfur dioxide (SO_2), which can be generated endogenously in mammals, has been found to have vasorelaxation activity, improve cardiac function and decrease myocardial injury. Endogenous SO_2 also mediates the process of fibrosis. Inhibition of endogenous SO_2 can aggravate small pulmonary artery remodeling and abnormal collagen accumulation. SO_2 treatment significantly improves pulmonary fibrosis and pulmonary arterial remodeling. Overexpression of the key enzymes associated with endogenous SO_2 generation, aspartate aminotransferase (AAT) 1 and AAT2, mimics the effect of SO_2 on the down-regulation of collagen synthesis, while AAT1 or AAT2 knockdown aggravates abnormal collagen accumulation in vascular smooth muscle cells (VSMCs). SO_2 also improves myocardial fibrosis induced by myocardial infarction or diabetes in rats, and inhibits myocardial fibroblast proliferation and migration by the extracellular signal-regulated protein kinase pathway. The mechanisms underlying the inhibition of fibrosis by SO_2 are related to its antioxidant effect, anti-inflammation effect, improvement in cardiac function, and cell proliferation inhibition. Therefore, SO_2 has a potential therapeutic effect on fibrosis.

Key words: Sulfur dioxide, Fibrosis, Therapy

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Introduction

Tissue fibrosis is an important problem in clinical diseases and up to 45% of all deaths are due to fibrotic diseases in various organs (Wynn, 2008). When fibrosis occurs in organs, such as the lung, heart, liver and kidney, it will cause organ dysfunction and can even lead to death. The abnormal excessive and irregular deposition of extracellular matrices (ECM) is the key cause of fibrosis (Nogueira et al., 2017; Park et al., 2019; Weiskirchen et al., 2019). Fibroblasts are the principal cells involved in the production of ECM in fibrosis. ECM is composed of matrix proteins, such as collagen and elastin, structural glycoproteins (proteoglycans) and carbohydrates (hyaluronan) (Gressner and Weiskirchen, 2006; Tarbit et al., 2019). Collagens are the main component of ECM (Tarbit et al., 2019). Many pathological conditions (such as inflammation, hypoxia or hypertension, etc.) can induce the formation of pro-inflammatory cytokines, chemokines and angiogenic factors, which activate fibroblasts or mesenchymal cells to generate collagens and non-structural proteins in the ECM (Weiskirchen et al., 2019). However, no effective method for treating fibrosis has been developed.

A gasotransmitter is a small gaseous molecule,

endogenously generated, which exhibits special functionality at physiological concentrations, and has specific molecular or cell targets (Wang, 2002). Nitric oxide, carbon monoxide, hydrogen sulfide (H_2S), and sulfur dioxide (SO_2) are considered the four main gasotransmitters (Huang et al., 2016a; Yu et al., 2018). SO_2 is considered a toxic environmental gas. However, SO_2 is endogenously generated and plays an important role in mammal physiology. SO_2 can relax aortic rings and down-regulate blood pressure in rats (Du et al., 2008; Lu et al., 2012). SO_2 also improves cardiac function, reduces myocardial ischemia reperfusion, and decreases myocardial injury induced by isoproterenol (Liang et al., 2011; Wang et al., 2011). Additionally, SO_2 improves pulmonary vascular remodeling, and reduces pulmonary fibrosis and myocardial fibrosis (Yu et al., 2016; Wang et al., 2018). Further studies have shown that aspartate aminotransferase (AAT, a key enzyme in SO_2 generation) 1 or AAT2 overexpression can mimic the inhibitory effect of SO_2 on fibrosis, while AAT1 or AAT2 knock down aggravates fibrosis in pulmonary artery smooth muscle cells (Huang et al., 2016b). Therefore, SO_2 may be a potential therapy for fibrosis.

Endogenous SO_2 and its associated enzymes in fibrosis pathogenesis

SO_2 is endogenously generated by L-cysteine, then catalyzed by cysteine dioxygenase and converted to L-cysteine sulfinate, then transaminated by AAT, converted to beta-sulfinylpyruvate, and finally spontaneously decomposed to pyruvate and SO_2 (Singer and Kearney, 1956; Stipanuk, 1986). The content of SO_2 is 15.54±1.68 μmol/L and its key generating enzyme (AAT) activity is 87±18 U/L in plasma (Du et al., 2008). SO_2 and AAT are extensively found in arteries, including the aorta, pulmonary, mesenteric, tail, and renal arteries. SO_2 and AAT are also extensively found in other tissues, such as heart, brain, pancreas, lung, kidney, spleen and liver tissues (Luo et al., 2011). In pulmonary hypertension induced by monocrotaline in rats, the content of SO_2 and activity of AAT were significantly increased in lung tissues (Jin et al., 2008). An inhibitor of SO_2 production, L-aspartate-beta-hydroxamate (HDX) reduced the generation of SO_2 and activity of AAT in monocrotaline-treated rats, but aggravated pulmonary artery remodeling, and further increased the relative medium thickness and relative media area of small pulmonary arteries. Inhibition of SO_2 by HDX significantly increased the percentage of pulmonary muscularized arteries, enhanced the content of collagen I and III, and up-regulated the mRNA expression of pre-collagen I and III in lung tissues of hypoxic rats (Sun et al., 2010). Inhibition of SO_2 by HDX also aggravated myocardial fibrosis in streptozotocin-induced diabetic rats (Liu et al., 2017). Therefore, the endogenous SO_2/AAT pathway is involved in the process of fibrosis in pulmonary hypertension induced by hypoxia or monocrotaline in rats.

The effect of SO_2 on lung fibrosis

SO_2 improved lung fibrosis in monocrotaline-treated rats. The contents of collagen I and III, and hydroxyproline were increased in lung tissues of monocrotaline-treated rats, and increased following HDX treatment, but decreased after SO_2 treatment (Yu et al., 2016). The mRNA expression of procollagen I and III in lung tissues were significantly up-regulated in monocrotaline-treated rats, and were higher following HDX treatment, but decreased after SO_2 treatment. In addition, the protein expression of matrix metalloproteinase-13 (MMP-13) and tissue inhibitor of metalloproteinase-1 (TIMP-1) were also detected in lung tissues. In monocrotaline-treated rats, the expression of MMP-13 and TIMP-1 was significantly increased, and was higher following SO_2 treatment, but decreased after HDX treatment. The MMP-13/TIMP-1 ratio was also significantly increased in monocrotaline-treated rats, and was higher following SO_2 treatment, but decreased after HDX treatment (Yu et al., 2016). Therefore, endogenous SO_2 in lung fibrosis in monocrotaline-treated rats, as well as exogenous SO_2, reduces abnormal collagen accumulation in lung tissue. In addition, SO_2 regulates the balance between MMP-13 and TIMP-1, increases collagen degradation, and limits pulmonary fibrosis.

The effect of SO_2 on vascular remodeling

Vascular remodeling often occurs alongside hypertension. In pulmonary hypertension induced by monocrotaline in rats, the relative medium thickness and relative median area of the medial and small pulmonary arteries were significantly increased (Jin et al., 2008). However, they decreased following treatment with SO_2 (Na_2SO_3/$NaHSO_3$, 72.3 mg/kg subcutaneously injected every day for three weeks). Therefore, SO_2 ameliorates vascular remodeling. A similar effect for SO_2 was also found in pulmonary hypertension induced by hypoxia in rats. SO_2 significantly decreased the high percentage of muscularized arteries induced by hypoxia (Sun et al., 2010). In hypoxia- or monocrotaline-treated rats, the content of collagen I and III, and the mRNA expressions of pre-collagen I and III were all significantly increased in lung tissues, but decreased after treatment with SO_2 (Sun et al., 2010; Yu et al., 2016). The mRNA expressions of MMP-13 and TIMP-1 were also significantly up-regulated in small and medium pulmonary arteries of hypoxia rats, and decreased following treatment with SO_2. The ratio of MMP-13 to TIMP-1 mRNA obviously decreased in hypoxia rats, but increased following treatment with SO_2 (Sun et al., 2010). MMP-13 stimulated the degradation of collagen, and TIMP-1 inhibited this degradation. The imbalance between MMP-13 and TIMP-1 contributes to vascular remodeling. Therefore, the inhibitory effect of SO_2 on pulmonary artery remodeling is related to the inhibition of abnormal collagen synthesis and induction of collagen degradation.

In cultured VSMCs under the conditions of AAT1 or AAT2 overexpression, the level of endogenous SO_2 and SO_2 in the supernatant were both significantly increased (Huang et al., 2016b). The protein expressions of collagen I and III were highly increased by transforming growth factor (TGF)-β1 in VSMCs, and even increased following AAT1 or AAT2 knockdown, but decreased due to AAT1 or AAT2 overexpression. The mRNA expressions of procollagen I and III were also significantly increased by TGF-β1 in VSMCs, and further increased by AAT1 or AAT2 knockdown, but decreased by AAT1 or AAT2 overexpression (Huang et al., 2016b). Additionally, the mRNA and protein expressions of MMP-13 were significantly decreased by TGF-β1 in VSMCs, and further decreased by AAT1 or AAT2 knockdown, but increased by AAT1 or AAT2 overexpression. The mRNA and protein expression of TIMP-1 were significantly up-regulated by TGF-β1 in VSMCs, and increased by AAT1 or AAT2 knockdown, but decreased by AAT1 or AAT2 overexpression. Therefore, the beneficial effect of SO_2 on vascular remodeling is related to inhibition of collagen synthesis and the promotion of collagen degradation.

SO_2 can also inhibit VSMC proliferation induced by serum (Liu et al., 2014). The higher expression of proliferating cell nuclear antigen (PCNA) can be significantly down-regulated by AAT1 or AAT2 overexpression, but increased in serum-induced VSMCs. Exogenous and endogenous SO_2 repressed cell proliferation, but did not affect cell apoptosis of serum-induced VSMCs. In platelet-derived growth factor (PDGF)-BB treated VSMCs, the high expression of cyclin D1 induced by PDGF-BB was inhibited by AAT1 or AAT2 overexpression, and increased by AAT1 or AAT2 silencing (Liu et al., 2014). SO_2 inhibited the phosphorylation of extracellular regulated protein kinase (Erk) 1/2 (Thr202/Tyr204), mitogen-activated protein kinase (MAPK) kinase 1/2 (Ser217/221) and the phosphorylation of RAF proto-oncogene serine/threonine-protein kinase (c-Raf, Ser338) induced by PDGF-BB. SO_2 also promoted the cyclic adenosine monophosphate/protein kinase A (cAMP/PKA) pathway and inhibited activation of c-Raf. A selective inhibitor of PKA, H-89, reversed the inhibitory effect of SO_2 on proliferation. Therefore, SO_2 inhibits the proliferation of VSMCs by down-regulation via the cAMP/PKA pathway.

SO_2 reduces cardiac fibrosis

Recent studies also showed that SO_2 alleviates cardiac fibrosis in myocardial infarction rats or diabetic rats (Liu et al., 2017; Wang et al., 2018). In myocardial remodeling induced by myocardial infarction in rats, the internal diameter of the left ventricle was increased, and the left ventricle wall became thinner (Wang et al., 2018). The study found that SO_2 treatment significantly increased the thickness of the wall, and decreased the enlarged internal diameter of the left ventricle. SO_2 reduced the area of cardiac fibrosis, and reduced the higher contents of myocardial collagen I and III induced by myocardial infarction. Additionally, in rats with myocardial infarction, the activity of MMP-9 was increased, but TIMP-1 was decreased. SO_2 treatment decreased the activity of MMP-9 and increased the level of TIMP-1. The MMP-9/TIMP-1 ratio was increased in rats with myocardial infarction, but decreased following SO_2 treatment (Wang et al., 2018). In addition, SO_2 significantly reduced myocardial fibrosis in streptozotocin-induced diabetic rats, while the SO_2 synthase inhibitor, HDX, aggravated myocardial fibrosis (Liu et al., 2017). SO_2 also decreased the expression of MMP9, MMP24 and TIMP1 in myocardial tissues, but increased these factors in the HDX-treated groups. Therefore, endogenous SO_2 mediates the process of myocardial fibrosis in streptozotocin-induced diabetic rats. SO_2 reduces cardiac fibrosis by reducing abnormal collagen accumulation and promoting collagen degradation.

Mechanisms of SO_2 in fibrosis

Fibrosis is the abnormal accumulation and deposition of ECM, including collagen and elastin, structural glycoproteins and carbohydrates (Weiskirchen et al., 2019). Pathological injuries provoke inflammatory cell infiltration and activation, and generate many harmful molecules, such as reactive oxygen species and inflammatory and fibrogenic cytokines. These molecules stimulate fibroblast or mesenchymal cell activation and produce a large amount of ECM. However, SO_2 has been shown to reduce these injurious effects of fibrosis development (Fig. 1). Studies have demonstrated that SO_2 exerts antioxidant and anti-inflammatory activity, reduces collagen synthesis, induces collagen degradation, and inhibits cell apoptosis during fibrosis development (Sun et al., 2010; Jin et al., 2013b; Li et al., 2016; Yu et al., 2016; Yang et al., 2018). Signaling pathways are also involved in the inhibitory effect of SO_2 in fibrosis (Huang et al., 2016b).

SO_2 and hypertension

Hypertension can cause mechanical stretching of vascular tissues and plays an important role in fibrosis. Blood pressure was significantly increased in spontaneously hypertensive rats, but decreased following SO_2 treatment (Lu et al., 2012). In addition, SO_2 administration significantly decreased the high systolic pulmonary artery pressure in pulmonary hypertension induced by abdominal aorta-inferior vena cava shunting (Luo et al., 2013). SO_2 also decreased the mean pulmonary artery pressure induced by monocrotaline in rats (Jin et al., 2008). Additionally, SO_2 treatment significantly decreased high mean arterial pressure in D-gal-induced aging rats (Dai et al., 2018). Inhibition of endogenous SO_2 by HDX caused even higher mean pulmonary artery pressure in pulmonary hypertension

induced by monocrotaline in rats (Jin et al., 2008). These data indicate that endogenous SO_2 is involved in the development of pulmonary hypertension and that the decrease in hypertension by SO_2 is related to its vasorelaxation effect. SO_2 can dose-dependently (1–12 mmol/L) relax isolated aortic rings and may be a physiologic endothelium-derived relaxing factor (Du et al., 2008; Meng et al., 2012; Wang et al., 2017b).

SO_2 and cardiac function

SO_2 improves cardiac function, which may contribute to an improvement in cardiac fibrosis. In diabetic rats with myocardial fibrosis, SO_2 improved cardiac function, increased ejection fraction and fractional shortening, and decreased left ventricular end-diastolic dimension (LVEDD) and left ventricular end-systolic dimension (LVESD) (Liu et al., 2017). In rats with cardiac dysfunction induced by cecal ligation and puncture, mean arterial blood pressure, left ventricular systolic pressure and maximum contraction velocity were significantly decreased, but these parameters were increased after SO_2 treatment (Yang et al., 2018). Left ventricular end-diastolic pressure was also increased in these rats, but decreased after SO_2 treatment. Therefore, SO_2 improves cardiac function in rats with sepsis-induced cardiac dysfunction. In rats with myocardial injury induced by isopropylarterenol, SO_2 treatment reversed the inhibition of left ventricular ejection fraction and fraction shortening, and reduced the anterior wall thickness of the diastolic left ventricle (Jin et al., 2013a). In addition, SO_2 reduced the high protein expression of GRP78, caspase-12 and CHOP (endoplasmic reticulum stress markers) induced by isopropylarterenol in myocardial tissues (Chen et al., 2012). In isolated rat hearts with ischemia reperfusion injury, cardiac function was improved by SO_2 preconditioning, but was reversed by 2-(2-Amino-3-methoxyphenyl)-4H-1-benzopyran-4-one (PD98059, an inhibitor of the ERK1/2 signaling pathway) (Huang et al., 2013). Therefore, the ERK1/2 signaling pathway mediates the beneficial effect of SO_2 preconditioning on cardiac function in isolated rat hearts with ischemia reperfusion injury.

SO_2 and fibroblasts

SO_2 is also involved in collagen synthesis by fibroblasts. In cultured pulmonary artery fibroblasts, mechanical stretching was induced by a computer-controlled cyclic strain unit. The results showed that mechanical stretching decreased the content of SO_2 in the supernatant, and reduced the protein expression of AAT1 and activity of AAT in primary pulmonary artery fibroblasts (Liu et al., 2016). However, the expressions of collagen I and III were significantly up-regulated. AAT1 knockdown mimicked these effects of mechanical stretching. In contrast, AAT1 overexpression reversed the effects of mechanical stretching described above, increased the protein expression of AAT1 and activity of AAT, increased the concentration of SO_2 in the supernatant, and down-regulated the expression of collagen I and III in primary pulmonary artery fibroblasts. Mechanical stretching also increased the expression of TGF-β1 and phosphorylation of Smad2/3, which were inhibited by AAT1 overexpression and

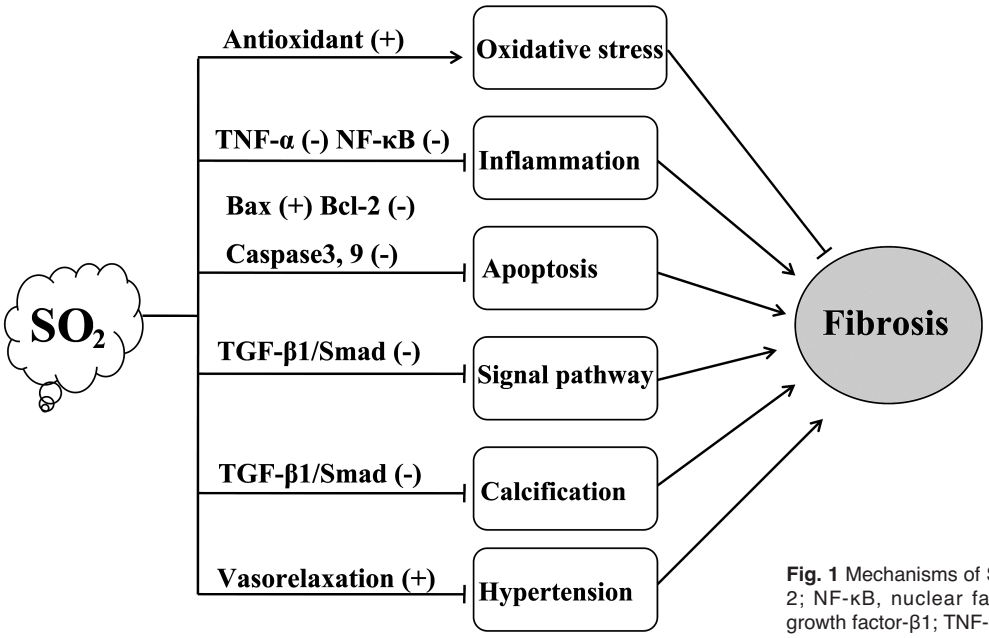

Fig. 1 Mechanisms of SO_2 in Fibrosis. Bcl-2, B-cell lymphoma-2; NF-κB, nuclear factor-kappa B; TGF-β1, transforming growth factor-β1; TNF-α, tumor necrosis factor-α.

enhanced by AAT1 knockdown. SB431542, an inhibitor of the TGF-β1/Smad2/3 pathway, reversed the effect of increased collagen I and III expression induced by AAT1 knockdown (Liu et al., 2016). Therefore, the effect of mechanical stretching on abnormal collagen accumulation is mediated by the SO_2/AAT1 pathway via the TGF-β1/Smad2/3 signaling pathway.

Empty lentivirus or AAT knockdown lentivirus was used to determine the role of endogenous SO_2/AAT in rat myocardial fibroblasts (Zhang et al., 2018b). The study found that the expression of AAT was reduced by 79% in AAT1 knockdown myocardial fibroblasts, and the production of SO_2 was reduced by 84.18% in the cell supernatant (Zhang, et al., 2018b). The expression of AAT was reduced by 77% in AAT2 knockdown myocardial fibroblasts, and the production of SO_2 was reduced by 82.49% in the cell supernatant. Therefore, an endogenous SO_2/AAT pathway exists in rat myocardial fibroblasts. In AAT1 or AAT2 knockdown rat myocardial fibroblasts, the indicator of proliferation, PCNA expression and the activity of CCK-8 were significantly enhanced, and cell migration was also promoted. SO_2 treatment reduced the high expression of PCNA and CCK-8 activity, and inhibited cell migration in AAT1 or AAT2 knockdown fibroblasts. The phosphorylation of extracellular receptor kinase was highly expressed in AAT1 or AAT2 knockdown lentivirus transfected myocardial fibroblasts. An inhibitor of the MAPK pathway, PD98059, inhibited increased fibroblast proliferation and migration induced by AAT1 or AAT2 knockdown (Zhang et al., 2018b). Therefore, the effect of SO_2 on myocardial fibroblast proliferation inhibition is related to down-regulation of the ERK signal pathway.

SO_2 and oxidative stress

Oxidative stress is involved in the process of fibrosis and is considered to be a therapeutic target for pulmonary fibrosis (Fois et al., 2018; Hosseinzadeh et al., 2018). In pulmonary hypertension induced by monocrotaline in rats, SO_2 increased antioxidative enzymes (such as plasma SOD and glutathione peroxidase [GSH-Px]) in lung tissues and plasma (Jin et al., 2008). The effect of SO_2 on the improvement in pulmonary vascular remodeling may be related to a reduction in oxidative stress. In acute lung injury induced by oleic acid, SO_2 significantly reduced the formation of oxygen radicals (OH^- and H_2O_2), decreased lipid peroxidation (malondialdehyde, MDA), and enhanced the antioxidant content (total antioxidant capacity, catalase, SOD and GSH-Px) in rat lungs. The protein expression of SOD1 and SOD2 in lung tissues was also up-regulated by SO_2 treatment (Chen et al., 2015). SO_2 also increased the activities of myocardial SOD and GSH in isoproterenol-induced rat myocardial injury, up-regulated the mRNA expression of SOD2 and GSH-Px1, and decreased the formation of H_2O_2 and $O2^{\bullet-}$ (Liang et al., 2011). A similar effect of SO_2 was also found in myocardial ischemia reperfusion injury. In rats with myocardial ischemia reperfusion injury, SO_2 preconditioning increased the levels of SOD, GSH and GSH-Px in plasma, and increased the expression of SOD1 in myocardium, but decreased the formation of MDA (Jin et al., 2013a). SO_2 also reduced the increased content of H_2O_2 and MDA in the aorta of D-galactose (D-gal)-induced aging rats, and enhanced the activity of SOD (Dai et al., 2018). Therefore, SO_2 enhances the activities of antioxidative enzymes, decreases free radical formation, and reduces oxidative stress.

SO_2 and inflammation

Inflammation is also involved in pulmonary vascular structural remodeling in pulmonary hypertension induced by hyperoxia (Sun et al., 2010). The expression of nuclear factor-kappa B (NF-κB) and intercellular adhesion molecule 1 (ICAM1) were increased in the pulmonary arteries of hypoxic rats, but significantly decreased following SO_2 treatment (Sun et al., 2010). In EA.hy926 cells or primary endothelial cells, with cystathionine-γ-lyase (CSE, a key enzyme in H_2S generation) knockdown, the generation of SO_2 was increased and the activity of AAT was enhanced. An H_2S donor (sodium hydrosulfide, NaHS) reversed these effects of CSE knockdown, and reduced the generation of SO_2 and AAT activity (Zhang et al., 2018a). Therefore, the generation of SO_2 can be inhibited by endogenous H_2S. In CSE knockdown primary human umbilical vein endothelial cells, the expression of ICAM1 and the phosphorylation ratios of inhibitor of NF-κBα (IκBα) and NFκB p65 were increased. Interleukin (IL) 6 and tumor necrosis factor (TNF)-α were also increased in the supernatant (Zhang et al., 2018a). HDX treatment increased the phosphorylation of NFκB p65 and the formation of inflammatory cytokines. A similar effect of SO_2 was also found in monocrotaline-treated rats. The SO_2 content and AAT activity were increased in lung tissues in monocrotaline-treated rats, but down-regulated in those rats treated with NaHS. Expression of the inflammatory factor, ICAM1, and the levels of IL6 and TNF-α were increased, but H_2S production was decreased in lung tissues of monocrotaline-treated rats. HDX treatment increased the protein expression of ICAM1, IL6 and TNF-α in lung tissues of monocrotaline-treated rats. Therefore, increased SO_2 generation plays a compensatory role to inhibit inflammation induced by H_2S/CSE deficiency.

SO_2 can also regulate inflammation in adipose tissues. SO_2 and AAT are extensively found in adipose tissues, including perivascular, aorta, heart, lung and liver adipose tissues (Zhang et al., 2016). In cultured 3T3-L1 adipocytes infected by the AAT1 adenovirus, the generation of SO_2 and the protein expression of AAT1 were significantly increased. AAT1 overexpression significantly reduced the high level of monocyte chemoattractant protein-1 (MCP-1) and IL-8 induced by TNF-α in the cell supernatant. AAT1 overexpression

also reduced the phosphorylation of NF-κB p65 and inhibited the degradation and phosphorylation of IκBα in 3T3-L1 adipocytes treated with TNF-α. This effect was reversed by lentivirus delivered shRNA, which caused AAT1 knockdown. AAT1 knockdown increased the phosphorylation of NF-κB p65, and aggravated the phosphorylation and degradation of IκBα. The generation of SO_2 and the protein expression of AAT1 were down-regulated by AAT1 knockdown. High levels of MCP-1 and IL-8 in the supernatant of 3T3-L1 adipocytes were promoted by TNF-α. Inhibitors of NF-κB activation (pyrrolidine dithiocarbamate or Bay 11-7082) successfully reversed this effect of AAT1 knockdown (Zhang et al., 2016; Chen et al., 2017). The NF-κB p65 pathway may mediate the effect of AAT1 deficiency on MCP-1 and IL-8 secretion. Therefore, SO_2 inhibits adipocyte inflammation induced by TNF-α.

SO_2 and apoptosis

Cell apoptosis also plays important roles in the process of fibrosis. In spontaneously hypertensive rats, SO_2 treatment significantly increased the apoptosis index, down-regulated the expression of Bcl-2, and up-regulated the expressions of Fas and caspase-3 in aortic tissues (Zhao et al., 2008). In addition, the proliferation index of VSMCs was inhibited. Therefore, the effect of SO_2 on alleviating structural remodeling is related to the induction of apoptosis and inhibition of smooth muscle cell proliferation. On the other hand, in rats with myocardial injury induced by isopropylarterenol, SO_2 treatment significantly reduced the high percentage of TUNEL-positive cells, and decreased the activities of caspase-3 and caspase-9 in left ventricular tissues (Jin et al., 2013b). SO_2 also increased the inhibition of protein expression of B-cell lymphoma-2 (Bcl-2), and decreased the increased protein expression of Bax induced by isopropylarterenol in myocardial tissues. SO_2 preconditioning also reduced apoptotic cardiomyocytes and decreased the apoptotic index in rats with myocardial ischemia reperfusion injury (Wang et al., 2011). SO_2 preconditioning (1–10 μmol/kg) reduced neuronal injury and decreased neuronal apoptosis in rats with febrile seizures (Han et al., 2014). SO_2 also reduced cardiomyocyte apoptosis, decreased the expression of myocardial Bax, caspase-3 and caspase-9, and increased the expression of Bcl 2 in streptozotocin-induced diabetic rats (Liu et al., 2017). SO_2 significantly reduced apoptotic cardiomyocytes, decreased the high Bax/Bcl-2 ratio and activity of caspase-3 in sepsis rats induced by cecal ligation and puncture (Yang et al., 2018). SO_2 also reduced hippocampal necrotic and apoptotic cells in cerebral ischemia reperfusion injury by increasing the antioxidant enzymes, SOD and glutathione (Zare Mehrjerdi et al., 2018). However, in epileptic rats, inhibition of SO_2 delayed the occurrence of apoptosis, but did not prevent apoptosis of neurons (Niu et al., 2018). The protein expression of PCNA was inhibited by overexpression of AAT1, and up-regulated by AAT1 knockdown in cultured pulmonary artery smooth muscle cells or pulmonary artery fibroblasts (Yu et al., 2016). SO_2 stimulated the apoptosis of pulmonary artery smooth muscle cells, but did not affect pulmonary artery fibroblasts.

Signaling pathways involved in SO_2 effects on fibrosis

The TGF-β1/Smad pathway is involved in the process of vascular fibrosis. The protein expression of TGF-β1 was significantly up-regulated in lung tissues of monocrotaline-treated rats, and increased following HDX treatment, but significantly decreased after SO_2 treatment (Yu et al., 2016). The expression of TGF-β1 was also significantly increased in pulmonary arteries of monocrotaline-treated rats as shown by immunohistochemical staining, but decreased following SO_2 treatment. The protein expressions of collagen I and III were significantly increased by TGF-β1 treatment in cultured primary rat pulmonary artery fibroblasts, but these effects were reversed by SO_2 treatment. SO_2 also inhibited the high phosphorylation of Smad2/3 and serine phosphorylation of TGFβ receptor I kinase (TβRI) induced by TGF-β1 in pulmonary artery fibroblasts (Yu et al., 2016). AAT1 overexpression increased AAT activity and the content of SO_2 in the supernatant, inhibited the high phosphorylation of Smad2 and Smad3, and inhibited the high content of collagen I and III induced by TGF-β1 in cultured pulmonary artery fibroblasts. In contrast, AAT1 knockdown inhibited AAT activity and the content of SO_2 in the supernatant, aggravated the high phosphorylation of Smad2 and Smad3, and further increased the levels of collagen I and III induced by TGF-β1 in cultured pulmonary artery fibroblasts. Therefore, the TGF-β1/Smad pathway is involved in the alleviation of pulmonary vascular fibrosis by SO_2.

AAT1 or AAT2 overexpression inhibited the high phosphorylation of Smad2 and Smad3 induced by TGF-β1 in VSMCs, and reduced the mRNA expression of plasminogen activator inhibitor-1 (Huang et al., 2016a,b). In contrast, AAT1 or AAT2 knockdown aggravated the high phosphorylation of Smad2/3 induced by TGF-β1. Additionally, the high phosphorylation of TβRI induced by TGF-β1 was inhibited. AAT1 or AAT2 overexpression was aggravated by AAT1 or AAT2 knockdown. These data indicate that endogenous SO_2 inhibited the phosphorylation of Smad2/3 and TβRI in collagen remodeling of VSMCs induced by TGF-β1. Furthermore, SB431542, an inhibitor of the TGF-β1/Smad pathway, decreased the high mRNA and protein expression of procollagen I and III induced by AAT1 or AAT2 knockdown. Therefore, the effect of SO_2 on inhibiting collagen remodeling is mediated, to some extent, by the TGF-β1/Smad signaling pathway. The protein expression of TGF-β and phosphorylation of Smad2/3 were significantly increased in rats with aortic

calcification, but decreased following SO_2 treatment (Li et al., 2016). SO_2 also inhibited the expression of Runx2 (osteochondrogenic marker), and decreased the high protein expression of TGF-β and phosphorylation of Smad2/3 induced by $CaCl_2$ in cultured VSMCs (A7r5 cells) (Li et al., 2016). Therefore, the effect of SO_2 on reducing rat vascular calcification is related to the TGF-β1/Smad pathway.

SO_2 treatment significantly improved aortic endothelium-dependent relaxation induced by acetylcholine (Dai et al., 2018). In addition, SO_2 treatment significantly decreased the high level of angiotensin I, and reduced the protein overexpression of angiotensin II type 1 receptor in thoracic aorta tissues in D-gal-induced aging rats. Therefore, the attenuating effect of SO_2 on endothelial dysfunction is related to inhibition of the angiotensin II/angiotensin II type 1 receptor pathway in D-gal-induced aging rats. In rats with pulmonary hypertension induced by high pulmonary blood flow, SO_2 significantly increased the content of H_2S in lung tissues, and increased the expression of key enzymes involved in H_2S generation, such as cystathionine-γ-lyase, mercaptopyruvate transsulfurase and cystathionine-β-synthase in small pulmonary arteries as shown by immunostaining (Luo et al., 2013). Therefore, SO_2 up-regulated the endogenous H_2S pathway in rats with pulmonary hypertension induced by high pulmonary blood flow. SO_2 can also down-regulate increased protein expression of p-Akt1/2/3, up-regulate protein expression of caspase-3 and E-cadherin, and down-regulate mRNA expression of DKK1 in the Wnt pathway in the lung tissues of rats with hypoxia-induced pulmonary hypertension (Luo et al., 2018). These findings indicate that SO_2 can inhibit the proliferation of pulmonary artery smooth muscle cells and improve pulmonary arteriolar remodeling via the Dkk1/Wnt signaling pathway.

Perpectives and challenges

Fibrosis is a pathological feature of most chronic diseases and causes organ dysfunction. It is characterized by excessive accumulation and deposition of ECM. The imbalance between collagen synthesis and degradation plays a key role in fibrosis. SO_2 is generated endogenously and endogenous SO_2 mediates fibrosis development. Inhibition of endogenous SO_2 by HDX aggravates small pulmonary artery remodeling and increases abnormal collagen accumulation. AAT1 or AAT2 knockdown aggravates abnormal collagen accumulation in VSMCs. On the other hand, SO_2 treatment significantly alleviates pulmonary fibrosis and pulmonary vascular remodeling. SO_2 decreases abnormal collagen accumulation and induces collagen degradation. The mechanism by which SO_2 attenuates fibrosis is related to its antioxidant effect, anti-inflammation effect, beneficial effect on cardiac function, and inhibitory effect on pulmonary artery smooth muscle cell proliferation.

However, the following limitations are associated with this study, (1) Fibrosis can occur in different organs and this study only demonstrated the effect of SO_2 in the lung, heart and vascular system. The effects of SO_2 on other organs (such as liver, kidney, and intestine) require further investigation. (2) The SO_2 donors used in this study were Na_2SO_3/$NaHSO_3$, and other donors such as SO_2 water solution were not studied. (3) There is a network of gasotransmitters (such as nitric oxide, carbon monoxide and hydrogen sulfide) in mammals. SO_2 plays a compensatory role to inhibit inflammation induced by H_2S/CSE deficiency (Zhang, et al., 2018a). It is unknown whether there is cross-talk and the relationship between SO_2 and other gasotransmitters in fibrosis is unclear.

SO_2 drugs can be activated to release SO_2 (Pardeshi et al., 2018; Wang and Wang, 2018), and SO_2 prodrugs have been shown to have an antimycobacterial effect and to inhibit methicillin-resistant Staphylococcus aureus (Malwal et al., 2012; Pardeshi et al., 2015; Wang et al., 2017a). The effectiveness and safety of SO_2 prodrugs require further study. In future studies, we will determine the effects and mechanisms of SO_2 on fibrosis in other organs. Suitable doses for SO_2 prodrugs also need further investigation. Thus, further experimentation is required to determine the potential therapeutic effect of SO_2 in fibrosis.

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