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Endocannabinoid 2-arachidonoylglycerol protects inflammatory insults from sulfur dioxide inhalation via cannabinoid receptors in the brain

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ABSTRACT

Sulfur dioxide (SO_2) pollution in the atmospheric environment causes brain inflammatory insult and inflammatory-related microvasculature dysfunction. However, there are currently no effective medications targeting the harmful outcomes from chemical inhalation. Endocannabinoids (eCBs) are involved in neuronal protection against inflammation-induced neuronal injury. The 2-arachidonoylglycerol (2-AG), the most abundant eCBs and a full agonist for cannabinoid receptors (CB1 and CB2), is also capable of suppressing proinflammatory stimuli and improving microvasculature dysfunction. Here, we indicated that endogenous 2-AG protected against neuroinflammation in response to SO_2 inhalation by inhibiting the activation of microglia and astrocytes and attenuating the overexpression of inflammatory cytokines, including tumor necrosis factor alpha (TNF-a), interleukin (IL)-1β, and inducible nitric oxide synthase (iNOS). In addition, endogenous 2-AG prevented cerebral vasculature dysfunction following SO_2 inhalation by inhibiting endothelin 1 (ET-1), vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule 1 (ICAM-1) expression, elevating endothelial nitric oxide synthase (eNOS) level, and restoring the imbalance between thromboxane A2 (TXA2) and prostaglandin I2 (PGI2). In addition, the action of endogenous 2-AG on the suppression of inflammatory insult and inflammatory-related microvasculature dysfunction appeared to be mainly mediated by CB1 and CB2 receptors. Our results provided a mechanistic basis for the development of new therapeutic approaches for protecting brain injuries from SO_2 inhalation.

Introduction

Sulfur dioxide (SO_2) is a key factor for air pollution in urban areas, which has been comprehensively studied due to the combustion of coal, power plants, and factories. These facts force us to revisit the adverse health outcomes of SO_2 pollution. Epidemiological investigations reveal that SO_2 pollution not only increases the risk of many respiratory diseases (Guo et al., 2014; Liu et al., 2014) but is also associated with circulatory system diseases (Amancio and Nascimento, 2012), such as heart disease (Li et al., 2015; Dong et al., 2015), congenital malformation (Farhi et al., 2014) and liver disease (Tong et al., 2015). Increasing evidence suggests that SO_2 inhalation is also linked to neurotoxicity and aggravates the risk for hospitalization and mortality of many brain disorders, including ischemic stroke (Amancio and Nascimento, 2014),

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cerebral hemorrhage (Ye et al., 2009), epilepsy (Cakmak et al., 2010), neurodevelopmental impairment and cognitive deficit (Lin et al., 2014). In the pathophysiology of these neurological diseases, a state of neuroinflammation accompanied by the alteration of the microvasculature function has been shown to be involved (Grammas et al., 2011; Farkas et al., 2000). In particular, our recent results confirm that acute SO_2 treatment causes neuroinflammation and microvasculature dysfunction, including the abnormal expression of inflammatory cytokines (inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2), and intercellular adhesion molecule 1 (ICAM-1) and endothelial dysfunction related factor (endothelin 1 (ET-1) and endothelial nitric oxide synthase (eNOS)), and exacerbates cerebral ischemic responses (Sang et al., 2010; Yun et al., 2010). Specifically, neuroinflammation following chronic SO_2 inhalation above the environmental standard has been reported to impair neuronal behavior and repress memory-related kinase activation and the gene expression of glutamate receptors (Yao et al., 2015). Taken together, these findings suggest that SO_2 exposure causes brain insults, and inflammation-related responses might be an important molecular modulator during this process.

Endocannabinoids (eCBs) have been reported to be involved in a variety of physiological, pharmacological and pathological processes as an endogenous signaling mediator (Marrs et al., 2010; Pertwee et al., 2010) and have the ability to bind to and functionally activate cannabinoid receptors (CB1 and CB2) to exert anti-inflammatory and neuroprotective properties against harmful insults (Mackie, 2006; Chen et al., 2011). As the most abundant eCBs and a full agonist for CB1 and CB2 receptors (Sugiura et al., 2006), 2-arachidonoylglycerol (2-AG) has been shown to play an important role in protecting neurons from inflammatory stimuli (Panikashvili et al., 2001; Kreutz et al., 2007). Subsequent experimental studies demonstrate that indirect elevation of endogenous 2-AG or direct application of 2-AG protected hippocampal neurons from the adverse effects of interleukine-1 beta (IL-1β) and lipopolysaccharide (LPS), β-amyloid stimuli and other brain harmful insults by weakening the inflammatory reactions (Chen et al., 2011; Nomura et al., 2011). Brain microvascular dysfunction is an important determinant of the inflammatory response to brain injury. The experimental evidence shows that neuroinflammation participates in brain microvascular dysfunction (Wu et al., 2012). Interestingly, 2-AG also affects other physiological processes, such as vasculature dysfunction and platelet function (Kvasnicka, 2010; Bouchard et al., 2003). 2-AG abolishes the toxicity effect of ET-1-induced cerebral microvascular responses via CB1/2 receptors (Mechoulam and Shohami, 2007). Thus, we hypothesized that endocannabinoid 2-AG may play a protective role against inflammatory insults in the brain and following microvascular dysfunction in response to SO_2 inhalation via CB1/2 receptors.

2-AG is mainly produced from diacylglycerol (DAG) by diacylglycerol lipase (DGL) and is hydrolyzed to arachidonic acid (AA) by monoacylglycerol lipase (MAGL) (Nomura et al., 2011). Thus, MAGL inhibition can elevate endogenous 2-AG level. Whereas, JZL184 is a selective inhibitor of MAGL and has been used for disrupting MAGL and improving the endogenous 2-AG content. In the present study, we established sub-acute SO_2 inhalation models (14 mg/m^3, 6 hr/day, 7 days) in the absence or presence of pre-intraperitoneal injection of JZL184 and investigated the protection of 2-AG against brain neuroinflammation and proinflammatory cytokines-mediated microvascular dysfunction following SO_2 inhalation.

1. Material and methods

1.1. Experimental animals and exposure treatments

C57BL6 mice, weighing 18–20 g, were purchased from the Center of Experimental Animal of Hebei Province and used according to the guidelines approved by the Institutional Animal Care and Use Committee of Shanxi University. The mice were divided randomly into 5 equal groups, in which each group consist of 8 animals, including vehicle, SO_2, JZL184 (a monoacylglycerol lipase inhibitor, Cayman, USA) + SO_2, SR-1 (SR141716, a CB1 antagonist, Cayman, USA) + JZL184 + SO_2, SR-2 (SR144528, a CB2 antagonist, Cayman, USA) + JZL184 + SO_2 groups. Similarly, vehicle and SO_2 groups were intraperitoneally injected with normal saline, and other groups were intraperitoneally injected with JZL184 (12 mg/kg), SR-1 (5 mg/kg) + JZL184, SR-2 (5 mg/kg) + JZL184 for 7 days, respectively. All reagents were injected once every two days for a total of three times in a 7-day timespan. Mice were then treated with SO_2 (14 mg/m^3) for 6 hr/day (10:00 a.m. to 16:00 p.m.) for 7 days. To obtain the desired concentration and generate SO_2 gas for distribution among the entire chamber, the SO_2 exposure appliance was prepared as previously described (Yao et al., 2015). In addition, the concentration of SO_2 exposure in the chamber was monitored by applying a real-time SO_2 monitor (FIX660, Wandi, China). An alkali absorption device was used to absorb the gas waste. During the SO_2 exposure process, the mice were allowed free movement under normal circumstances, except for access to food and water. The 1-hr China's Ambient Air Quality Standard Grade II of SO_2 is 0.5 mg/m^3 (GB 3095–2012, 2012). The concentration used in this study was 14 mg/m^3 (6 hr/day), then the 24-hr average concentration every day

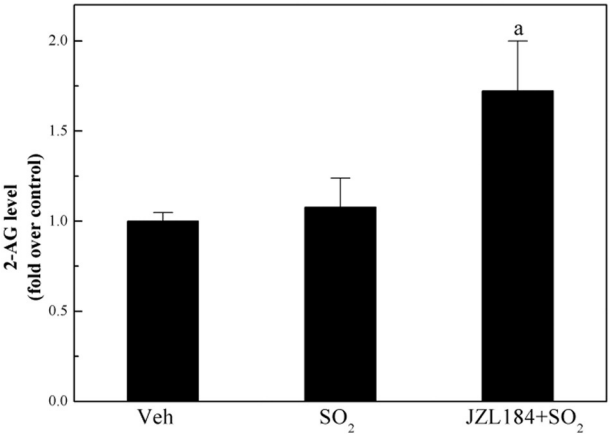

Fig. 1 – JZL184 improves the 2-AG level in mice brain after SO_2 exposure. The level of 2-AG in the brain of mice under different treatment conditions (n = 8). $^a p < 0.05$ compared with vehicle controls. 2-AG: 2-arachidonoylglycerol; SO_2: sulfur dioxide.

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