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Hydrogen sulfide inhalation-induced immune damage is involved in oxidative stress, inflammation, apoptosis and the Th1/Th2 imbalance in broiler bursa of Fabricius

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ABSTRACT

Hydrogen sulfide (H_2S) is widely accepted to be a signaling molecule that exhibits some potentially beneficial therapeutic effects at physiological concentrations. At elevated levels, H_2S is highly toxic and has a negative effect on human health and animal welfare. Studies have shown that H_2S exposure induces an immune function in mice, but there are few studies of the effect of continuous H_2S exposure on immune organs in poultry. In this study, one-day-old broilers were selected and exposed to 4 or 20 ppm of H_2S gas for 14, 28 and 42 days of age. After exposure, the bursa of Fabricius (BF) was harvested. The results showed that continuous H_2S exposure reduced the body weight, abdominal fat percentage, and antibody titer in broilers. H_2S exposure also decreased mRNA expression of IgA, IgM and IgG in the broiler BF. A histological study revealed obvious nuclear debris, and a few vacuoles in the BF, and an ultrastructural study revealed mitochondrial and nuclear damage to BF cells after H_2S exposure for 42 d. Terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling (TUNEL) assay suggested H_2S exposure remarkably increased the number of TUNEL positive nuclei and significantly increased apoptotic index. The expression of apoptotic genes also confirmed that H_2S inhalation damaged the broiler BF. Increased cytokines and reduced antioxidant responses were detected in the BF after exposure to H_2S. Cytokines promoted inflammation and caused a Th1/Th2 imbalance. We suggest that continuous H_2S intoxication triggers oxidative stress, inflammation, apoptosis and a Th1/Th2 imbalance in the BF, leading to immune injury in broilers.

1. Introduction

Hydrogen sulfide (H_2S) is a colorless irritant gas that smells like rotten eggs. H_2S is a highly reactive and toxic xenobiotic gas that is found at a high level in the environment and is a signaling molecule at physiological concentrations (Szabo, 2018). H_2S is released from pulp and paper mills, natural gas production, animal containment and manure handling, and geothermal power plants (Lancia et al., 2013; Malone et al., 2017). It has been reported that the yearly H_2S emissions from all sources on land reach 27–150 million metric tons (Chou, 2003). H_2S is both an environmental pollutant and a hazard in more than 70 occupational settings (Jr et al., 2005; Rumbeiha et al., 2016). For instance, an H_2S concentration greater than 250 ppm (ppm) leads to sudden death, loss of consciousness, and pulmonary edema (Frame and Schandl, 2015; Strickland et al., 2003). The agency for toxic substances and disease registry in America summarized the toxicological effects of H_2S: it causes respiratory damage, neurological damage, myocardial damage, and reproductive system damage (Chou et al., 2016). The U.S. government considers H_2S to be a high priority chemical threat in industry and a potential weapon of mass destruction that can be used by terrorists due to its ability to paralyze olfactory receptors and hide its presence (Mustafa et al., 2009). Among all sources, animal feeding

Abbreviations: ADG, average daily gain; Bax, bcl-2-like protein 4; Bcl-2, b-cell lymphoma 2; BF, bursa of Fabricius; BW, body weights; Caspase-3, 8, 9, cysteine-aspartic proteases 3, 8, 9; DFI, daily feed intake; FC, feed/gain, feed conversion ratio; GSH, glutathione; GSH-Px, glutathione peroxidase; H_2O_2, hydrogen peroxide; H_2S, hydrogen sulfide; HI, hemagglutination-inhibition; IFN-γ, interferon gamma; IL-1β, 4, 6, 10, 12, interleukin 1β, 4, 6, 10 and 12; MDA, mitochondrial malonaldehyde; ND/NDV, Newcastle disease/Newcastle disease virus; NO/iNOS, nitric oxide/inducible nitric oxide synthase; ppm, parts per million; qRT-PCR, quantitative real-time PCR; ROS/RNS, reactive nitrogen/oxygen species; SOD, superoxide dismutase; T-AOC, total antioxigenic capacity; Th1/Th2, type I/II helper T cells; TNF-R_1, tumor necrosis factor receptor 1; TNF-α, tumor necrosis factor alpha; TUENL, terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling

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operations are the most commonly studied, especially in swine and poultry containment (Guarrasi et al., 2015), which reflects the propensity of H_2S poisoning of animals and regulators (Malone et al., 2017). H_2S in livestock is one of the most important factors that influences animal well-being and their production performance, and H_2S also increases the risk potential of farm workers. The concentration of H_2S, which is released from organic decomposition, in modern poultry houses and swine containment facilities will reach approximately 10.0 ppm and 100.0 ppm, respectively (Guarrasi et al., 2015). The concentration is much higher in winter, when ventilation systems are working inefficiently, and in old containment systems in which litter is not cleaned in time. Almost every year in rural Middle America, farm workers die of acute H_2S exposure in the hog industry (Rumbeiha et al., 2016).

H_2S exposure has a negative effect on animal growth characteristics (Dorman et al., 2004; Saillenfait et al., 1989; Wang et al., 2011) and also damages the immune response. For example, a study indicated that H_2S significantly impaired the antibacterial system in rats, consequently compromising their immune responses (Rogers and Ferin, 1981). Rabbit alveolar macrophage exposure to H_2S (54 ppm) for 24 h reduced the percentage of alveolar macrophages and changed the cellular ultrastructural morphology (Robinson, 1982). Chicken is an important experimental animal that has been well-characterized in many biological aspects and bridges the evolutionary gap between mammals and other vertebrates (Hillier et al., 2004). The sensitive nature of birds to the environment is used as an important method of environmental monitoring (Chen et al., 2018). However, the effect of H_2S on the chicken immune function, especially the molecular mechanism, has not been elaborated detailly.

The bursa of Fabricius (BF) is a primary humoral immune organ that is responsible for the establishment and maintenance of the B cell compartment in avian species (Murthy and Ragland, 1992). This organ serves as an essential site for the generation of antibody diversity by gene conversion. This organ consists of 98% B lymphocytes (Olah, 2014), but there is infiltration of other cells, such as T cells and macrophages (Abdul-Careem et al., 2008). B cells mediate protection against pathogens by presenting antigens and secreting antibodies and by producing cytokines that regulate the quality and magnitude of the humoral and cellular immune responses. For example, tumor necrosis factor (TNF)-α production by B cells is necessary for sustained antibody production. The cytokine microenvironment also determines Th polarization from naïve T cells to certain subtypes of Th cells. Interleukin (IL)-12 p40 and interferon (IFN)-γ trigger type I helper T (Th1) cells differentiation, and IL-4 promotes Type II helper T (Th2) cells (Wojciechowski et al., 2009). These cytokines not only cause inflammation and affect the Th1/Th2 ratio. A Th1/Th2 imbalance could lead to immune disorder, inducing dendritic cell maturation and the formation of lymphoid structures or impairing antigen presenting cell function and pro-inflammatory cytokine release by monocytes. Many environmental factors can change the levels of cytokine expression. For example, sulfur dioxide markedly elevated the IL-4 level and decreased the IFN-γ level in asthmatic rats (Li et al., 2014). H_2S induced inflammation in rat epithelial cells both in vivo and in vitro by regulating IL-6 and IFN-γ (Beaumont et al., 2016; Chou et al., 2016). However, it is unclear whether H_2S alters cytokine expression in the chicken BF. Additionally, both acute and chronic inflammatory states in vivo are coupled with significant alterations of the redox equilibrium due to the associated enhancement of oxidant generation (Li et al., 2013; Rochette et al., 2013). A study suggested that H_2S increased the mitochondrial malonaldehyde (MDA) content and decreased mitochondrial superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activity in nerve cells (Li et al., 2012). Hence, H_2S exposure induces oxidative stress. Both inflammation and oxidative stress are crucial factors that induce cell apoptosis. Meanwhile, apoptosis plays a critical role in the immune response, contributing to many pathologies. For example, it was demonstrated that the amount of apoptosis was proportional to the severity of the clinical disease of Newcastle disease (ND) (Harrison et al., 2011). Recent studies reported that H_2S induced apoptosis in various cell types, including human lung fibroblasts (Baskar et al., 2006) and pancreatic acinar cells (Cao et al., 2006). Khan et al. also showed that alveolar macrophage cells exposed to 400 ppm H_2S showed significantly decreased numbers of viable cells (Khan et al., 1991). These data suggested that H_2S could induce immune cell apoptosis, but these findings must be confirmed.

Despite increasing evidence indicating that H_2S exposure induces oxidative stress, inflammation and apoptosis, the effect of H_2S on chicken immune tissue is still unclear. In this study, we evaluate the impact of H_2S inhalation on oxidative stress, cytokines and apoptosis in the broiler BF. These data will provide valuable clues for H_2S inhabitation-induced immune impairment and provide a reference for comparative medicine.

2. Materials and methods

2.1. Animals and treatment

All procedures used in this experiment were approved by the Institutional Animal Care and Use Committee of Northeast Agricultural University. A total of 200 one-day-old Ross 308 broilers were obtained from a commercial hatchery (Yinong Poultry Industry Co., Ltd., Harbin, China). Birds were randomly divided into two groups and raised in two different separate environmentally controlled chambers as the control group or H_2S group. The atmospheric H_2S gas (4.0 ± 0.5 ppm, 1–21 days of age; 20.0 ± 0.5 ppm, 22–42 days of age) and treatments of birds in H_2S group are shown in Fig. S1. The same procedures were carried out in the control group except the concentration of H_2S, which is maintained at 0.5 ± 0.5 ppm. The concentration of H_2S was maintained using a H_2S decompressor (Dawn Gas Co. Ltd., Harbin, China) and monitored with a Luma Sense Photoacoustic Field Gas-Monitor Innova-1412 (Santa Clara, CA, USA). Birds in each room were housed in 5 cages containing equal numbers of chicks (20) per cage and provided with water and feed ad libitum. Chicks were fed commercial broiler diets according to the recommendations of the National Research Council (Dale, 1994). The room temperature was maintained at 33 ± 1 °C for the first 3 d. After 3 days, the temperature was gradually reduced by 3 °C per week until reaching 24 °C at the end of the experiment. During the experiment, the lighting program was 23 h light: 1 h dark during the first week and was then changed to 18 h light: 6 h dark using fluorescent lights. Temperature and airflow were controlled during the exposures to ensure adequate ventilation, minimize buildup of animal-generated contaminants (NH_3, dander, carbon dioxide) and avoid thermal stress.

2.2. Sample collection, growth performance and BF index

Individual body weights (BW), average daily gain (ADG), daily feed intake (DFI) and feed conversion ratio (feed/gain; FC) were measured biweekly. Cumulative DFI was calculated by subtracting the remaining feed weights in the feeders from the initial feed-added in the feeders. At 14, 28 and 42 days of age, 10 chicks per treatment (2 chicks per cage) were randomly chosen and sacrificed using cervical dislocation. The BF was removed and weighed, and the BF index was calculated as the weight of the organ (g)/body weight (kg). After weighing, samples were rinsed with ice-cold sterile deionized water, frozen immediately in liquid nitrogen and stored at − 80 °C until required. At 42 days of age, 5 chicks in each group were chosen and cervically dislocated. The whole net carcass rate, dressing percentage and abdominal fat percentage were recorded. After harvesting the BF, the samples were separated into two parts, and half of the specimens were fixed in 10% buffered neutral formalin and the other samples were fixed with 2.5% for histological and ultrastructural observations.