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Original Research Article

New insights into the role of spermine in enhancing the antioxidant capacity of rat spleen and liver under oxidative stress

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ABSTRACT

Oxidative stress can damage cellular antioxidant defense and reduce livestock production efficiency. Spermine is a ubiquitous cellular component that plays important roles in stabilizing nucleic acids, modulating cell growth and differentiation, and regulating ion channel activities. Spermine has the potential to alleviate the effects of oxidative stress. However, to date no information is available about the effect of spermine administration on antioxidant property of the liver and spleen in any mammalian *in vivo* system. This study aims to investigate the protective effect of spermine on rat liver and spleen under oxidative stress. Rats received intragastric administration of either 0.4 μmol/g body weight of spermine or saline once a day for 3 days. The rats in each treatment were then injected with either diquat or sterile saline at 12 mg/kg body weight. Liver and spleen samples were collected 48 h after the last spermine ingestion. Results showed that regardless of diquat treatment, spermine administration significantly reduced the malondialdehyde (MDA) content by 23.78% in the liver and by 5.75% in the spleen, respectively ($P < 0.05$). Spermine administration also enhanced the catalase (CAT) activity, anti-hydroxyl radical (AHR) capacity and glutathione (GSH) content by 38.68%, 15.53% and 1.32% in the spleen, respectively ($P < 0.05$). There were interactions between spermine administration and diquat injection about anti-superoxide anion (ASA), AHR capacity, CAT activity, GSH content, and total antioxidant capacity (T-AOC) in the liver and about ASA capacity and T-AOC in the spleen of weaned rats ($P < 0.05$). Compared with the control group, spermine administration significantly increased the AHR capacity, CAT activity, GSH content, and T-AOC by 40.23%, 31.15%, 30.25%, 35.37% in the liver, respectively ($P < 0.05$) and increased the T-AOC by 8% in the spleen of weaned rats ($P < 0.05$). Compared with the diquat group, spermine + diquat group significantly increased ASA capacity by 15.63% in the liver and by 73.41% in the spleen of weaned rats, respectively ($P < 0.05$). Results demonstrate that spermine administration can increase the antioxidant capacity in the liver and spleen and can enhance the antioxidant status in the spleen and liver under oxidative stress.

1. Introduction

Reactive oxygen species (ROS) are generated during physiological processes and xenobiotic exposure in living organisms. Reactive oxygen species can be considered beneficial or harmful to organisms depending on their concentration. At physiologically low levels, ROS functions as a “secondary messenger” in intracellular signaling and regulation; however, excess ROS can result in oxidative stress (Circu and Aw, 2010). Oxidative stress can cause adverse damage to cellular macromolecules such as nucleic acids,

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proteins and lipids (Brieger et al., 2012), and intestine cells (Kim et al., 2012). Moreover, oxidative stress can affect the normal function of the immune system (Wang et al., 2010), reduce nutrient absorption and metabolism, and depress growth performance (Yuan et al., 2007). Oxidative stress is also related to a number of health disorders, including inflammatory disease, cancer (Reuter et al., 2010), cardiovascular, diabetes (Jomova and Valko, 2011), neurological (Jomova et al., 2010), and many other diseases. A previous study has suggested that substances such as vitamin C, vitamin E, and carbohydrates can suppress oxidative stress (Ryan et al., 2010; Kadian and Garg, 2012). Thus, supplementing components or food with antioxidant abilities to animals can be an effective approach to reduce oxidative stress (Devasagayam et al., 2004).

Spermine, a novel small molecule substance, is distributed in many living organisms such as animals, plants, some fungi, and some bacteria (Pegg and Michael, 2010) and plays important roles in myriad mechanism, including cellular proliferation and differentiation, gene transcription and translation (Pegg, 2014), modulation of ion channel function, cellular signal (Rao et al., 2012), and macromolecular synthesis (Igarashi and Kashiwagi, 2010). Previous experiments have shown that spermine administration can induce the maturation of villus and crypt cell function in jejunum and ileum (Buts et al., 1993). Further studies have revealed that spermine administration can significantly increase the specific activities of disaccharidase (e.g., maltase) in jejunum and enhance the intestinal absorption of macromolecules (Sugita et al., 2007; Cao et al., 2015). Therefore, spermine has been receiving considerable attention as a nutritional substance for accelerated functional maturation of the small intestines (Ramani et al., 2014). Furthermore, spermine acts as a free radical scavenger (Ha et al., 1998), a biologically important antioxidant *in vitro* (LØvaas and Carlin, 1991; Guerin et al., 2001; Shoji et al., 2005; Rider et al., 2007; Toro-Funes et al., 2013), and an anti-inflammatory agent (LØvaas and Carlin, 1991). Spermine administration can enhance the jejunum antioxidant properties of suckling rats (Cao et al., 2015) and serum antioxidant capacity of suckling piglets (Fang et al., 2016a), and alleviate serum oxidative stress in weaned rats (Liu et al., 2014). Therefore, spermine has potential functions against oxidative stress.

The liver is the main detoxifying organ in the body; this organ possesses a high metabolism rate and is prone to much damage potentially caused by oxidative stress. Thus, a correct status of the hepatic antioxidant defense system is significantly important for health maintenance. The spleen is one of the most important immune organs in the body and is mainly responsible for making antibodies, differentiating B cells, regulating immune responses, filtering aging erythrocytes, storing blood, and initiating immune reactions to blood-borne antigens. The normal structure and function of immune organs are connected with animal immunity. Oxidative damage caused by oxidative stress often leads to alteration in the structure and function of numerous organs (Azadzoi et al., 2005). Therefore, maintaining balance in the antioxidant defense system of the liver and spleen is very important for livestock breeding. However, to date no information is available about the effect of spermine administration on antioxidant property of the liver and spleen in any mammalian *in vivo* system. Diquat is a common herbicide, whose toxicity is related to disturbance of the total antioxidant capability of the body, and is widely used to cause oxidative stress in animal models such as rats and piglets (Abdollahi et al., 2004; Mao et al., 2014; Liu et al., 2016). Therefore, diquat was intraperitoneally injected to induce oxidative stress in the present study.

This study is part of a larger study that involved determining the metabolic profiles of spermine against oxidative stress (Liu et al., 2014). This study aims to explore the effects of spermine on the antioxidant status in rat liver and spleen under oxidative stress. The results can provide scientific evidence of the capacity of spermine to modulate antioxidant status and may pave the way for spermine development as a functional feed additive.

2. Material and methods

2.1. Experimental material

Weaned male Sprague–Dawley (SD) rats and their food were provided by Dossy Experimental Animals Co., Ltd. (Chengdu, China). Spermine (S3256-1G) and diquat (45422-250 mg-R) was obtained from Sigma Chemical Co. (St. Louis, MO, USA). Catalase (CAT), anti-superoxide anion (ASA), glutathione (GSH), malondialdehyde (MDA), total superoxide dismutase (T-SOD), total antioxidant capacity (T-AOC), anti-hydroxyl radical (AHR) and protein detection kits were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). All antioxidant parameters were measured by colorimetric analysis at the corresponding wavelength by multifunctional microplate reader SpectraMax M5 (San Francisco, USA) according to the reagent specification.

2.2. Experimental design and feeding management

The animal procedures for this study were approved by the Care and Use of Laboratory Animals of Sichuan Agricultural University, and followed the Guide for the Care and Use of Laboratory Animals established by the National Research Council. All rats were placed in individual metabolic cages and acclimatized to experimental conditions 1 day before starting the experiment. Forty 21-day-old weaned male SD rats weighing 38 to 45 g were randomly assigned to 4 treatments (10 rats per treatment): control, spermine, diquat, and spermine + diquat. The rats received intragastric administration of either 0.4 μmol/g body weight of spermine (spermine was dissolved in physiological saline) or sterile saline per day for 3 days. Subsequently, half of the saline-received rats were intraperitoneally injected with diquat at 12 mg/kg body weight, whereas the other half was injected with the same volume of sterile saline. The spermine-received rats were also divided into 2 groups (diquat-injection or sterile saline injection). The liver and spleen were immediately removed after ether anesthesia 24 h after the diquat injection. The tissues were washed in cold saline (0.9% NaCl; 4°C), frozen in liquid nitrogen, and then transferred to storage at −80°C until analysis. Rats had access to food and water *ad libitum*. The experimental conditions throughout the experiment were maintained at a temperature ranging from 22 to 25°C, a humidity between 50% and 70%, and a cycle of 12 h light/12 h dark.

2.3. Biochemical assays

2.3.1. Sample preparation

The sample was prepared using the method of Zhang et al. (2008). Approximately 0.1 g of sample (liver or spleen) was quickly weighed, thawed, and homogenized in 10 volumes (wt/vol) of ice-cold normal saline (0.7 g/mL). The homogenates of sample were centrifuged at 6,000 × *g* for 10 min at 4°C. The supernatant was acquired and stored at −20°C for biochemical analysis.

2.3.2. Protein content assay

The protein content of spleen and liver was determined using the method described by Georgiou et al. (2008) using a protein analysis kit (Coomassie Brilliant Blue), and bovine serum albumin as the protein standard. The sample preparation of protein is in accordance with the previously described in sample preparation