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Sulfur dioxide inhalation lowers sperm quality and alters testicular histology via increasing expression of CREM and ACT proteins in rat testes

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ABSTRACT

Sulfur dioxide (SO_2) is one of the main atmospheric pollutants worldwide, and is reported to be responsible for the formation of severe haze in China. Some studies have demonstrated a potential harmful effect of SO_2 on the male reproductive system; however the underlying mechanism is still unknown. The purpose of this study is to investigate the roles of cytochrome P450 (P450), cAMP-responsive element modulator (CREM), and activator of CREM (ACT) in SO_2-induced toxicity. Forty-eight male Wistar rats were randomly divided into an experimental and control group. The experiment group was exposed to SO_2 in ambient air (10 ppm, 4 h/day), and the control group was treated with filtered air in the same conditions. After 2 weeks, the results showed a significant decrease in body weight and sperm motility, and an increase in the testis weight-to-body weight ratio as compared to the control group. Histological investigation suggested that SO_2 exposure led to loose arrangement of the spermatogenic cells and local structural damage in the seminiferous tubules. Moreover, the expressions of P450, CREM and ACT proteins increased in the testes by 0.22%, 47.26% and 23.38%, respectively. Taken together, SO_2 inhalation lowered sperm quality, altered testicular histology, and increased expressions of CREM and ACT proteins in the testes of rats. Overall, these results could contribute to a better understanding of SO_2-induced male reproductive toxicity.

1. Introduction

Sulfur dioxide (SO_2) is a common acidic pollutant released from the burning of fossil fuels that causes a series of environmental problems (Bisseret and Blanchard, 2013). SO_2 was also reported to be responsible for the formation of the recent severe haze in China (He et al., 2014). Previous studies have indicated that SO_2 has a toxic effect on animals and humans, such as mitochondrial dysfunction, increased morbidity of cardiovascular diseases (Qin et al., 2016), changed lung capacity for healthy asthmatics and increased lung cancer (Qin et al., 2015). Additional studies have shown that the various chemical and physical substances derived from SO_2 in ambient air may have a detrimental impact on male fertility (Rengaraj et al., 2015). Meng et al. found that inhalation of SO_2 caused oxidative damage to the testes in mice (Meng and Bai, 2004). Our previous investigations further indicated that SO_2 treatment together with fluoride caused changes in sperm motility, serum testosterone levels, oxidative stress and metabolic enzyme activities in the testes of male rats (Zhang et al., 2006a,b). However, the underlying mechanisms remain unknown.

It is reported that cytochrome P450, cAMP-responsive element modulator (CREM), and activator of CREM (ACT) in testis, play a crucial role in the spermatogenesis and normal male reproductive function (Li et al., 2015; Mazaheri et al., 2014). For instance, changes in P450 aromatase enzyme activity and expression of cyp19a1 genes have been suggested to be major regulators in the production of gonadal 17β-estradiol (E2) during reproduction (Li et al., 2015). During spermatogenesis, CREM functions critically

Abbreviations: SO_2, sulfur dioxide; P450, cytochrome P450; CREM, cAMP-responsive element modulator; ACT, activator of CREM; CYPs, 17 cytochrome P450; E2, 17β-estradiol; CRE, response element; DAB, 3,3′-diaminobenzidine; CTRL, control; H.E, eosin hematoxylin.

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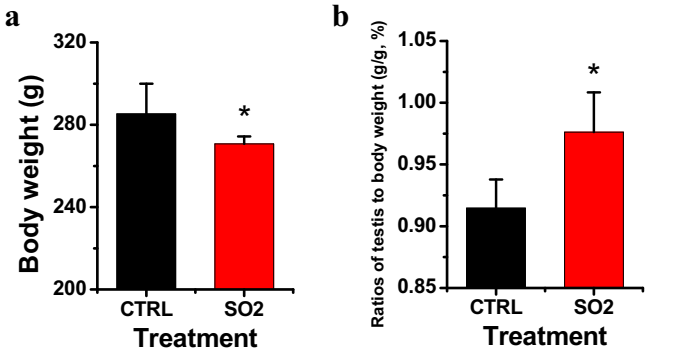

Fig. 1. The body weight (a) and testis to body weight ratio (b). Results are shown as mean ± S.D (n = 24). Asterisks indicate a statistically significant difference compared to controls (CTRL), * $p < 0.05$.

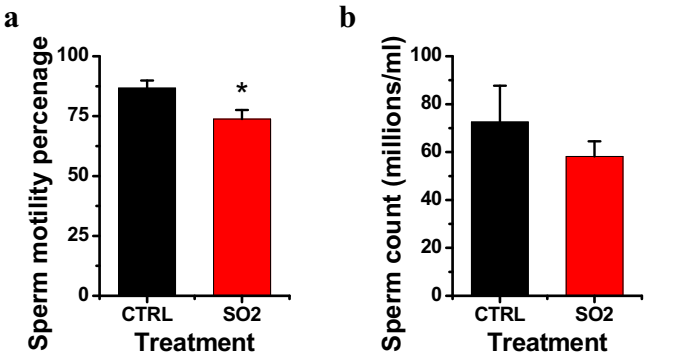

Fig. 2. The sperm motility percentage (a) and the sperm count (b) of rats. Results are shown as mean ± S.E (n = 6). Asterisks indicate a statistically significant difference compared to controls (CTRL), * $p < 0.05$.

in the phase after meiosis. As soon as spermatocytes completes meiosis, a number of highly specialized postmeiotic genes, including cAMP response element (CRE), perform crucial transcription activity downstream from cAMP (Sassone-Corsi, 2000). ACT is a tissue-specific factor in the testes and has a high affinity for CREM, promoting spermatogenesis (Grozdanov et al., 2016). However, the relationship between SO_2 exposure and P450, CREM, and ACT is unknown.

In the present study, in order to explore the possible mechanism of SO_2-induced spermatogenesis disorders, we assessed the sperm quality, testicular development and histology, and the expressions of P450, CREM, and ACT in the testes of rats after SO_2 treatment.

2. Materials and methods

2.1. Chemicals and antibodies

Pure SO_2 gas (99.99%) was provided by Foshan Kedi Gas Chemical Industry Co., Ltd, Guangdong, China, for use in this study. Rabbit anti-CYP11A1/P450SCC polyclonal antibody, SABC Staining Kit (AR1022), DAB kit was purchased from Boster Biological Technology (Wuhan, China). Rabbit anti-CYP11A1/P450SCC polyclonal antibody, was purchased from Boster Biological Technology (Wuhan, China); Rabbit polyclonal antibody specific for rat CREM and ACT was supplied by Abcam Trade (Shanghai) Company Ltd. All other chemicals used were of the highest commercially available grade.

2.2. Animals and treatment

Male Wistar rats (age 12 weeks, n = 48) were purchased from the Experimental Animal Center at Shanxi Medical University of China. Animals were housed in cages under standard conditions. After 1 week of adaptation, the rats were randomly divided into two groups. The treatment group was exposed to fresh air that contained 26.2 mg/m^3 (10 ppm) SO_2 in a lab-developed device (approved Chinese Patent, No.CN201110058955.9) for 4 h/day. The control group was exposed to fresh air under the same schedule in the same device. The concentration of SO_2 in the ambient air was monitored continuously with a detector (PGM-35, RAE Systems Inc., USA), and SO_2 tidal volume and inhalation concentration of rats were recorded. The rats had free access to food and water and the weight of animals were recorded weekly throughout treatment.

After two weeks, six rats were randomly selected from each group. Their epididymides were dissected to assess semen quality, while the testes were immediately removed and fixed with the Bouin's fixation solution for 12 h, dehydrated and embedded in paraffin.

2.3. Sperm count and sperm motility analysis

After removed from the rats, the epididymides were dipped into 1 ml normal saline solution preheated to 37 °C. The epididymis was cut into pieces in order to release the sperm into solution, and then the sperm solution was collected after small tissue pieces were removed. A 10 μl sample of sperm suspension was added onto a special glass slide and the slide was analyzed to determine sperm number and motility under a microscope following the method from our previous study (Zhang et al., 2006a).

2.4. Immunohistochemistry and image analysis

Serial sections were dewaxed, and endogenous peroxidase was inactivated by incubation in 30% fresh prepared hydrogen peroxide for 8 min at room temperature. The sections were then washed three times for 2 min with distilled water. Antigen was retrieved by 0.01 M citric acid buffer (pH 6.0) microwave antigen retrieval. The sections of antigen after repair were washed in PBS twice after cooling, followed by blocking in PBST solution containing 5% BSA for 30 min at room temperature in a wet-box. Next, sections were incubated with the primary anti-P450 rabbit monoclonal antibody for 1.5 h at 37 °C, anti-CREM rabbit monoclonal antibody for 3.5 h at 37 °C, and anti-ACT rabbit monoclonal antibody overnight at 4 °C. respectively, Then, the samples were washed with PBS for three times and inclcubuted by the horseradish peroxidase-conjugated goat-anti rabbit IgG for 20 min at 37 °C. After washing with PBS three times, the DAB (Boster, Wuhan, China) was used for coloration and sections were counterstained with hematoxylin for 5 min. Negative control sections were incubated with 0.1 M PBS instead of the primary antibody.

Ten visual fields per slide and 6 sections per male rats were selected randomly for analysis. Cells that contained yellow or brown yellow granules were regarded as the positive, while cells without yellow or brown yellow granules were considered negative. Optical densities of the positive cells from every view were measured by Image-Pro® Plus Version 5.1 micrograph analysis software (Media Cybernetics Inc. of America).

2.5. Statistical analysis

The data were analyzed by one-way ANOVA and *t*-test for significant difference between the control group and the SO_2 treatment group. Values were considered to be statistically significant with a p value under 0.05.