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Ameliorative effects of *N*-acetylcysteine on fluoride-induced oxidative stress and DNA damage in male rats' testis

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ABSTRACT

This study was to elucidate DNA damage in rats treated with sodium fluoride (NaF) by performing 8-Hydroxy-2-deoxyguanosine (8-OHdG) immunohistochemical staining assays on seminiferous tubules of rats' testis, and also to evaluate the protective effects of *N*-acetylcysteine (NAC) on spermatogenesis. Male Sprague Dawley (SD) rats were exposed to a single dose of NaF (25 mg/kg/day) with or without NAC (150 mg/kg/day) for 7 weeks (7W) by gastric gavage. Testicular fluorine content was detected by fluorine ion selective electrode method. Oxidative damage to DNA was evaluated by measuring the increase in 8-OHdG formation in testicular tissue through immunohistochemical staining assays and also the effects of NAC pretreatment. The biochemical indicators about oxidative stress were detected by colorimetric assays, sperm parameters and the morphological changes of testis were studied. NaF significantly increased serum levels of oxidative stress, markedly elevated testicular fluorine and 8-OHdG expression levels as well as the rate of sperm aberration compared to saline group. Testosterone in serum, sperm counts and the mobility of sperm were lower than those of the rats in control group. The pathological morphological changes in testicular seminiferous tubule were also obvious in the rats with NaF treatment. Pretreatment with NAC did not reduce the contents of fluoride content in testis, but significantly reduced 8-OHdG formation and lipid peroxidation. This study suggests that NAC may have certain antagonism on the reproductive damage induced by NaF.

1. Introduction

As we all know, excessive fluorine could induce skeletal toxicity, such as dental fluorosis and skeletal fluorosis [1]. Besides, excessive fluoride consumption could cause damage to non-skeletal tissue, such as brain, kidney, liver and testis, especially to male reproductive system. Animal experimental studies reported fluoride exerted an adverse effect on sperm quantity and quality, sperm chemotaxis [2], serum testosterone, follicle stimulating hormone and luteinizing hormone levels, spermatogenesis and steroidogenesis [3,4], testicular cell cycle [5]. Moreover, high doses of fluoride could result in apoptosis of Leydig cells [6], and vacuolar dystrophy in seminal cells and necrosis in mice [7]. Besides, excessive fluoride could induce disruption of reproductive hormones and low birth rates in humans [8,9]. Due to exposure to environmental pollutants and physical stress, the rate of male infertility has been increasing, which has become a very serious social problem [10,11]. Therefore, it is vitally significant to further investigate the molecular mechanism of reproductive toxicity induced by fluorosis and explore the proper antidote.

Currently, oxidative stress induced by NaF is still recognized to be associated with fluorosis formation. Researches suggested that fluorosis may cause excessive oxidative stress which impaired the balance of antioxidant system, hence induced oxidative damage to rat testes [12,13]. Recent study [14] showed that reactive oxygen species (ROS) caused the oxidation of DNA, proteins, and lipids, resulting in the formation of 8-OHdG and MDA. 8-OHdG is a product of oxidative damage following specific enzymatic cleavage after 8-hydroxylation of the guanine base, which has a biological role, in that it is capable of inducing G:C to T:A conversion during DNA replication. 8-OHdG could be used as a typical biomarker of oxidative DNA damage produced by ROS [15]. Some researches discovered that 8-OHdG was a biomarker of oxidative damage in benzopyrene carcinogenesis [16], diabetes mellitus [17], ageing [18]. Besides recent studies showed that 8-OHdG was a novel biomarker of inflammatory activity in patients with car-

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Table 1
Body weight and major organs coefficient after 7 weeks exposure ($n = 15$; mean ± SD).

Groups	Body weight (g)	Testis weight (g)	Epididymis weight (g)	Organs coefficient	
				Testis/body weight (%)	Epididymis/body weight (%)
Control	475.15 ± 34.77	1.71 ± 0.17	0.63 ± 0.05	0.36 ± 0.04	0.13 ± 0.01
NAC	472.23 ± 21.32	1.72 ± 0.16	0.61 ± 0.02	0.36 ± 0.03	0.13 ± 0.01
NaF	442.18 ± 31.06*	1.56 ± 0.12*	0.53 ± 0.07*	0.36 ± 0.03	0.13 ± 0.02
NaF+ NAC	459.54 ± 34.51	1.69 ± 0.18	0.59 ± 0.06	0.36 ± 0.05	0.13 ± 0.02

* $P < 0.05$, compared with the control group.

diac sarcoidosis [19]. Animal and human studies revealed that the prevalence of 8-OHdG-immunostained germ cells was positively correlated with clinical grades of varicocele [20,21]. Oxidative damage in testicular DNA is associated with poor semen quality, reduced fertility and increased risk of stillbirths and birth defects. Therefore, the study was conducted to evaluate the genetic toxic effect of NaF on male reproductive system by immunohistochemistry for 8-OHdG expression in testicular tissue, and speculate whether NaF could cause DNA damage.

N-Acetylcysteine (NAC) is widely used as a mucolytic agent and an antidote for acetaminophen overdose in the clinic. Besides, NAC could be involved in the regulation of oxidative stress related gene expression, hence it had an antagonistic effect on oxidative damage [22]. It was reported that NAC may prevent the exogenous toxicant-induced male reproductive injury through antagonizing oxidative damage [23,24]. However, the related domestic and foreign literatures about the regulating role of NAC in the NaF-induced male reproductive toxicity were few, and the specific mechanism of action was not clear yet.

The purpose of this study was to explore whether NaF induce oxidative stress and DNA damage in rats' testis, and examine the protective effects of antioxidant on the development of testicular dysfunction under such conditions. Through establishing animal model of sub-chronic fluorosis and using NAC as intervention agent, this research provided theoretical basis for revealing the pathogenetic mechanism of male reproductive toxicity induced by NaF and exploring the possible antidotes relieving fluorosis.

2. Materials and methods

2.1. Materials and chemicals

NaF and NAC were purchased from Sigma–Aldrich (St. Louis, MO, USA). Oxidative stress related biochemical indicator detection kits for SOD, CAT and MDA were provided by the Nanjing Jianchen Institute of Biotechnology (Nanjing, China). Serum testosterone ELISA test kit was purchased from ShangHai HengYuan Biological Technology Co., Ltd. (Shanghai, China). Rabbit anti-8-OHdG antibody was purchased from Bioss (Beijing, China). Computer-aided Semen Analysis System (CASA) was purchased from Hamilton Thorne Biosciences (Cummings Center, USA). PF—type 1 fluorine ion selective electrode was purchased from Shanghai Reunion Scientific Instrument Co., LTD (Shanghai, China). ELx808 Absorbance Reader was obtained from BioTek Instruments, Inc. (USA).

2.2. Animals and treatment

Sixty SD male specific pathogen free (SPF) rats that were 28 days old, weighing 100–150 g, were obtained from the Experimental Animal Center of Henan province (Zhengzhou, China) [License No. SCXK (Yu) 20100002]. Animals were housed in sterilized plastic cages with corncob bedding in a controlled-environment animal room (temperature, 23 ± 1 °C; relative humidity, 50 ± 10%; photoperiod, 12 h light/dark cycle). Free access to distilled water and sterilized food was allowed at all the time. Animals were allowed to acclimatize for the inspection and quarantine for 7 days prior to treatment. All animal procedures were performed in compliance with the regulations and guidelines of the international ethics committee on animal welfare.

In this experiment, 60 rats were randomly divided into four different treatment groups (15 rats per group). 0.09% physiological saline was used as solvent to prepare 2.5 g/L NaF solution and 15 g/L NAC solution, respectively. All solutions were administrated to rats in accordance with the principle of isovolumetric gavage (10 ml per kilogram of body weight) once per day for 7W. Animals in group 1(saline, saline control group) were administrated orally with saline(10 ml/kg) to determine the basal values for biochemical comparisons; the group 2 (NaF treated group) in which rats received a single dose of 2.5 g/L NaF, corresponding to 25 mg/kg/d, in order to induce sub-chronic fluorosis features; A single dose of 15 g/L NAC solution, corresponding to 150 mg/kg/d, was given to the third group (NAC treated group), in order to act as biochemical comparisons with the fourth group; the fourth group (the protective group, NAC + NaF) which received 150 mg/kg/day of NAC 0.5 h prior to oral NaF exposure (25 mg/kg/d). Since one spermatogenic cycle in the rat is 50 ± 2 days [25], in this way, we ensured that the fluoride-exposure duration contained at least a complete period of spermatogenesis in the rat. The symptom and mortality were observed and recorded carefully throughout the experimental schedule. Final body weights of the animals were recorded on the day of sacrifice after being anesthetized by chloral hydrate. Blood samples were obtained from abdominal aorta using vacuum tube after light anesthesia. Serum samples were separated by centrifugation and stored at −20 °C until determination of plasma testosterone. Testes and epididymides were dissected out and organ weights were measured by electronic balance. Sperms were quickly flushed out from the caudal part of both epididymides of each rat with 10 ml suspension medium and the resulting sperm suspension was used for determination of the quantity, the activity and malformation rate of sperm. Testes were frozen at −80°C.

2.3. Body weight, organ coefficient of testis and epididymis analysis

At the end of 7-week exposure, the body weights of all the rats were measured, the weights of testis and epididymis were measured when the rats were sacrificed and the organ coefficients of testis and epididymis were calculated according to the equation: organ coefficient = wet weight of organ (g)/body weight (g) × 100%.

2.4. Sperm quality assessment

Sperm from the cauda epididymis and vas deferens were allowed to disperse into HTF medium at 37 °C. Sperm were diluted to 5×10^6/ml in phosphate-buffered saline (PBS) and incubated at 37 °C, 5% CO_2 for further experiments. Direct smear of sperm suspension was conducted with computer-aided semen analysis system (CASA) to detect sperm count, sperm motility ratio. For sperm morphology, a drop of sperm suspension in medium was smeared on a slide, air dried, and fixed with methanol. After fix-