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Comparative Evaluation of the Effect of Sodium Nitrite on Reproductive Organ Weights and Sperm Count in Rats and Mice

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Sodium nitrite ($NaNO_2$) is a water soluble compound, well-known as a principal food preservative and colorant in the food industry. Besides the variety of industrial and medicinal applications, toxicity to humans and animals is well documented after nitrite overexposure. In the testis changes in hormonal profile and vascularisation have been reported. The current study aimed comparative assessment of early effects of acute $NaNO_2$ treatment on reproductive organ weights and indices in tandem with sperm count in rats and mice. Spermatozoa were isolated from both vas deferens and counted. An increase in testis weight and gonado-somatic index was found in tandem with reduction in epididymis weight and sperm count in both species following acute $NaNO_2$ treatment. Our comparative analysis on macro parameters of rat and mouse reproductive organs (testis and epididymis) and sperm counts suggest that mice are more vulnerable to the exposure to $NaNO_2$ than rats.

Key words: sodium nitrite, hypoxia, sperm count

Introduction

Sodium nitrite ($NaNO_2$) is an inorganic salt with various applications. It is widely used in the food industry as color fixative and preservative of fish and meat products (E250). It acts as a flavor-enhancer and retards rancidity by preventing fat oxidation. It also inhibits the growth of micro-organisms as *Clostridium botulinum* spores. Sodium nitrite is used for dye synthesis, manufacture of rubber chemicals and nitroso compounds and has several other industrial purposes. Medicinally, it is used for vasodilation, bronchodilation and as antidote for cyanide poisoning [3]. However, industrialization and uncontrolled use of nitrate/nitrite salts has increased human exposure to high levels of $NaNO_2$ which can act as a pro-oxidant and pro-carcinogen. Exposure to nitrite mainly occurs through the oral route. Nitrite taken through contaminated drinking water or food, primarily affects the gastrointestinal tract and small intestine [3]. Acute exposure to high levels of nitrite has been reported to cause death, mainly due to methemoglobinemia [4]. Chronic exposure to

lower doses of nitrite causes adverse health effects, which includes birth defects, respiratory tract ailments, damage to nervous system and paralysis. Prolonged exposure to nitrite can also cause carcinogenicity and mutagenicity [3]. Oxidative damage is considered to be one of the main mechanism by which nitrite exerts its toxicity. There is evidence of developmental and reproductive toxicity of $NaNO_2$ in experimental animal studies. In the testis changes in hormonal profile [6] and vascularisation have been reported [5]. Data about its influence on reproductive system are controversial. The current study aimed comparative assessment of the early effects of acute $NaNO_2$ treatment on reproductive organ weights and indices and sperm count in rats and mice.

Materials and Methods

The experiments were carried out on four-month-old male Wistar rats and two-month-old male ICR mice. Animals were maintained in the institute's animal house in standard hard bottom polypropylene cages at 23 ºC ± 2 ºC and 12:12 h light-dark cycle with free access to laboratory chow and tap water throughout the study. Sodium nitrite was injected intraperitoneally at a single dose of 50 $mg.kg^{-1}$ body weight for rats and 120 $mg.kg^{-1}$ body weight for mice. Treated animals were sacrificed at different time intervals following the administration (1h, 5h, 1d, 2d and 5d) under light diethyl ether anesthesia. The control animals were injected with distilled water. Testes and epididymides were sampled, weighed and their indices were calculated (organ weight to body weight ratio). Spermatozoa were isolated from both vas deferenses and counted using Buerker's chamber. Data were statistically processed using Student's t-test. The animal experiments were performed in accordance with the animal protection guidelines approved by the Ethics Committee for Experimental Animal Use at IEMPAM, BAS.

Results

Testis weight of rats was in normal range with slightly lower values at the 1st and the 5th hour, as well as on the first day after sodium nitrite administration. On the 2nd and the 5th day, this parameter reached control values (**Fig. 1**). Gonado-somatic index revealed more convincing tendency of significant increase by 13% compared to the control at the first hours (1h, 5h) and on the 5th day after treatment (**Fig. 1**).

In mice, there was slight and insignificant elevation by 7-10% in testis weight at the first hours (1h, 5h) and on the 1st and the 5th day after treatment (**Fig. 1**). This finding corresponded to significant elevation of mice gonado-somatic index by 20% - 35% at the same time points as in rats (**Fig. 1**).

Epididymis weight of rats was insignificantly lower at the 1st hour, followed by significant decrease by 20% at the 5th hour, compared to the control. Epididymis weight of mice revealed clear tendency of reduction in all time points investigated and the values were reduced by 30-50% from the control value (**Fig. 2**). Corresponding to these findings are the data on epididymis weight/body weight index in mice that revealed marked decrease by 35-40% compared to control value (data not shown).

Reduction in rat sperm count was observed in all the treatment groups that reached significance at most of the time points – by 30% at the 1st hour, by 47% at the 5th hour, by 28% on the 2nd day, and 22% on the 5th compared to the control (**Fig. 2**).

In mice we found reduction in sperm count in most of the treated groups with statistical significance on 2nd day after treatment (60% lower than the control value) (**Fig. 2**). At the 5th hour after treatment we estimated a significant elevation of the sperm count (30% higher compared to control).

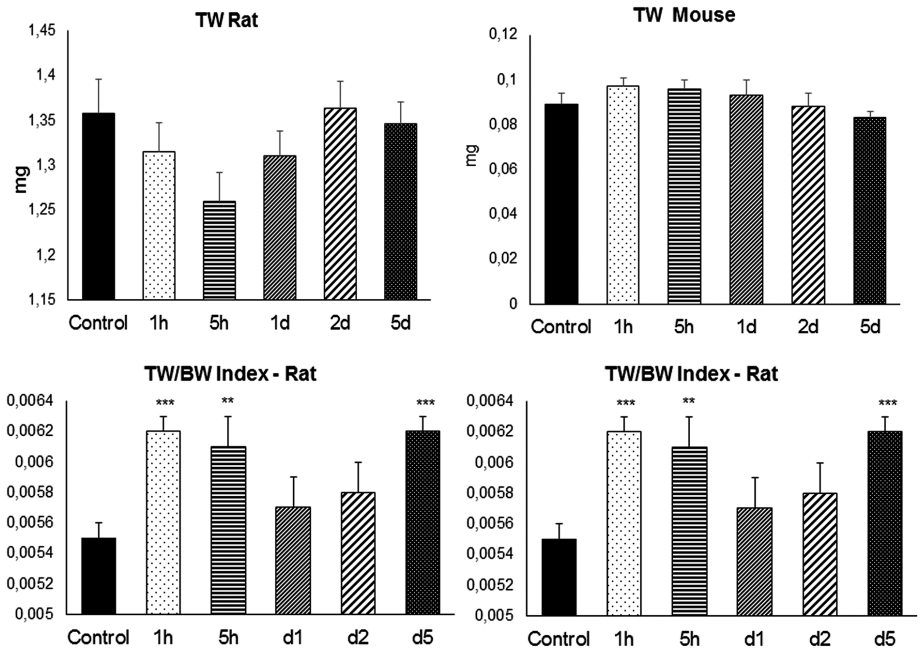

Fig. 1. Changes in testis weight (TW) and in gonado-somatic index (ratio of testis/body weight) at different time intervals following $NaNO_2$ treatment in rats and mice. Data represent mean value ± SE (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$)

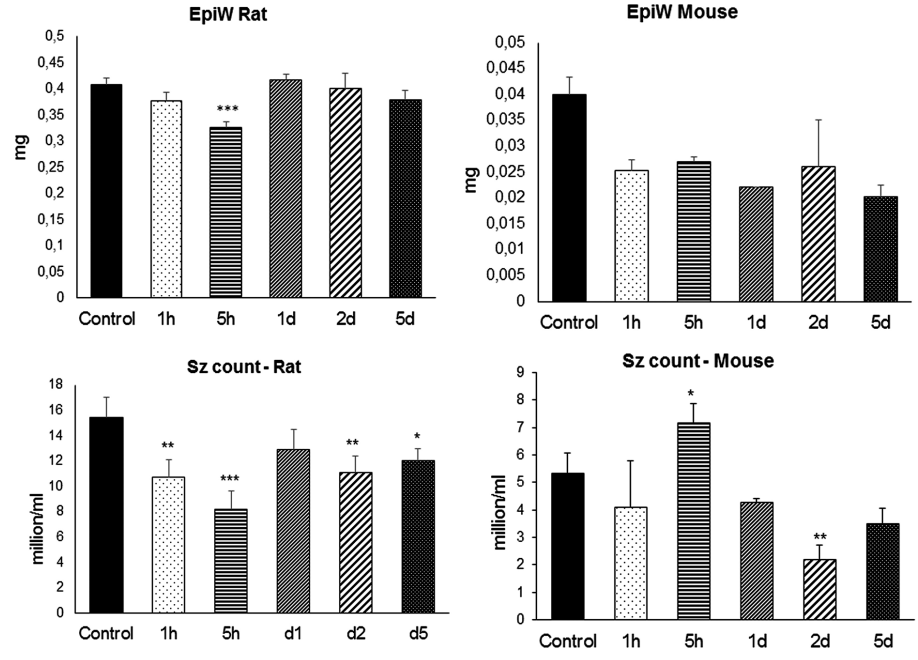

Fig. 2. Changes in epididymal weight and in sperm count at different time intervals following $NaNO_2$ treatment in rats and mice. Data represent mean value ± SE (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$). Sz - spermatozoa

Discussion

The toxic effect of nitrates and nitrites are well documented in mammalians, including impairment of reproductive function and hepatotoxicity. The major acute toxic effect of sodium nitrite intoxication is methemoglobinemia [10]. The over dosage of $NaNO_2$ leads to the accumulation of excess methemoglobin in the blood, which does not bind oxygen strongly, thus causing hemic hypoxia. The rate of methemoglobin formation varies between species, as well as with age of the organism, and the reaction is reversible. According to literature data, LD50 values of 85-220 mg of sodium nitrite per kilogram of body weight have been reported for mice and rats [13]. Because of the wide range of LD50 values and the species-specific rate of methemoglobin formation, we conducted experiments with graded doses of $NaNO_2$ in order to determine the optimal dose that induces acute toxicity and methemoglobinemia in both species without animal loss. At 50 mg of sodium nitrite per kilogram of body weight for the rats and 120 mg of sodium nitrite per kilogram of body weight for the mice the methemoglobin reached peak levels one hour after treatment (41%). Most of the mammals have little tolerance to hypoxia, and their response involves the activation of regulatory mechanisms at systemic, tissue, and cellular levels. Hypoxia has a decisive impact in different molecular pathways, which modulate several cellular functions, such as proliferation, apoptosis, angiogenesis, pH balance, and anaerobic glycolysis. The susceptibility of the mammalian testis to low oxygen pressure or content is a causative factor in some forms of male infertility [12]. In animal models (rat, mouse, guinea-pig, rabbit, monkey, sheep), it has been shown that hypobaric hypoxia induces partially reversible quantitative changes such as decrease in semen volume, sperm count and sperm motility [9]. Literature data for the effect of sodium nitrite-induced hemic hypoxia on the male reproductive system are controversial and uncompleted [8].

Our quantitative results in mice are more consistent than in rat. There was an increase in mouse testis weight up to 10% and in gonado-somatic index by 20-35% from the corresponding controls. In contrast, epididymis weight and ratio epididymis weight/body weight of mice were greatly reduced by 30-50%. With one exception at the 5^{th} hour after treatment, the mouse sperm count was decreased by 20-60% compared to control value. A possible explanation for elevation of testis weight and gonado-somatic index could be inflammatory process, based on data by Alyoussef et al. [1, 2] about enhanced gene and protein expression of pro-inflammatory cytokines (TNF-α, IL-1β, IL-6) after exposure to sodium nitrite. The authors have been reported elevation in testis weight and gonado-somatic index, as well. The elevation in testis weight and gonado-somatic index in tandem with reduction of epididymis weight and sperm count in our study on mice and rats could be interpreted as a result from retention of seminiferous tubules fluid together with sperms released into the tubular lumen. These findings might be a consequence form compromised hypothalamus-hypophysis-gonadal axis, in particular decreased serum testosterone levels associated with increased serum LH, FSH concentrations [1, 5]. Alternatively, prolonged residence of sperms in rete testis cannot be excluded.

We have established decrease in sperm count in all experimental groups in both species – in mice by 20-60% and in rat by 20-50% from corresponding controls. Our data are in agreement with the results by Alyoussef et al. [1, 2] who have demonstrated almost 50% reduction in the sperm count in tandem with increase in testis weight and gonado-somatic index after sodium nitrite administration. They also found significantly elevated levels of caspase-3, caspase-8 and caspase-9 activity associated with significant increase of cytotoxicity. Increased oxidative stress by $NaNO_2$ was believed to produce oxidation and damage to DNA leading to germ cell apoptosis. Our previous

histological findings on sodium nitrite-treated rats revealed destructive and degenerative changes in rat testis after sodium nitrite treatment [7]. We have found disorganized seminiferous tubules and sloughs of undifferentiated germ cells into the luminal area in some experimental animals. In some tubules the lumen has not be seen. Blood vessels with larger diameter were more frequently found compared to the control [7]. Tissue hypoxia of the male reproductive system with subsequent atrophy of germinal epithelium was associated with arrest of primate spermatogenesis [11].

Local changes in testicles exposed to hypoxia involved neovascularization and an increase in temperature as reported by Farias et al. [5]. Hyperthermia is well known to affect spermatogenesis leading to infertility. In this respect, the response of the testis to hypoxia (in particular, sodium nitrite-induced hemic hypoxia), could resemble other hyperthermia-related pathologies, such as varicocele and cryptorchidism.

In conclusion, our comparative analysis on macro parameters of rat and mouse reproductive organs (testis and epididymis) and sperm counts suggests that mice are more vulnerable to the exposure to $NaNO_2$ than rats.

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