CHANGES IN RAT TESTIS AND SPERM COUNT AFTER ACUTE TREATMENT WITH SODIUM NITRITE

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Abstract


Sodium nitrite (NaNO₂) is an inorganic salt with various industrial applications. The adverse health effects of NaNO₂ in animals and humans are typically due to the formation of methemoglobin in the blood. This can lead to cyanosis and, at very high levels, death. Humans are constantly exposed to sodium nitrite through food and drinking water. The aim of the present study is to follow up the changes in rat testis and sperm count after acute treatment with NaNO₂. Four-month-old male Wistar rats were intraperitoneally injected with NaNO₂ at dose of 50 mg.kg⁻¹ body weight (distilled water for controls). Treated animals were sacrificed at different time intervals (1 h, 5 h, 24 h and 48 h) following the administration. Testes and epididymides were sampled, weighed and embedded in paraffin using routine histological practice. Spermatozoa were isolated from both vasa deferentia and counted. Preliminary histological observations of the testis of some experimental animals demonstrated disorganization of seminiferous epithelium and assemblance of undifferentiated germ cells in the luminal area of the tubules. Testis weight/body weight index was increased in the first hours after administration, which is probably due to higher seminal fluid volume. Statistically significant reduction in sperm count ranging between 480 g.kg⁻¹ (fifth hour) and 280 g.kg⁻¹ (48 h) was observed. These results may be associated with impaired hormonal balance and tissue anoxia – an adverse environment for germ cell development. In conclusion, acute treatment with NaNO₂ affects testis morphology, some weight indices and sperm count in mature rats.

Key words: rat testis, sodium nitrite, sperm count

Abbreviations: MetHb – methemoglobin; ns – not significant; BW – body weight; TW – testis weight; EpiW – epididymal weight; Sz count – spermatozoa count

Introduction

Sodium nitrite (NaNO₂) is a water soluble, inorganic salt widely used in various industries including agricultural, chemical industry, textile processing industry, disinfectants, colouring agents, etc (U.S.DHHS, 2001). Humans are exposed to nitrate and nitrite through food and drinking water, with a minor contribution from the air. Sodium nitrite has been found to inhibit growth of disease causing microorganisms and it is a common food additive (E250) used as a color fixative and preservative in meats and fish. In blood, nitrite undergoes a coupled oxidation reaction with oxyhemoglobin to produce methemoglobin (MetHb). Unlike hemoglobin, MetHb cannot exchange oxygen; hence, the presence of excess MetHb in the circulation proportionately reduces the ability of the blood to transfer oxygen (RCHAS, 2000) and can cause hemic hypoxia. The body reacts to hypoxia with adaptive responses, such as relaxation of smooth muscle, an-
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Giogenesis and vasodilatation, thus increasing blood supply to tissues, compensating for the lack of oxygen (Farias et al., 2005a).

There are literature data mainly for the effect of hypobaric hypoxia at high altitudes on testis morphology and semen quality. The effect of NaNO2 and the subsequent hemic hypoxia are poorly investigated and data about its influence on reproductive system are controversial. The widespread use of NaNO2 in the food industry contributes to its potential health risk. This motivated us to investigate the effect of NaNO2 on rat spermatogenesis at different time intervals following administration.

Materials and Methods

The experiments were carried out on four-month-old male Wistar rats. The animals were divided into four sodium nitrite-treated groups (n = 15 rats per group) and age-matched control group (n = 16). Rats were maintained in the institute’s animal house in standard hard bottom polypropylene cages at 23ºC ± 2ºC 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 50 mg.kg⁻¹ body weight (one ml dosing volume). Treated animals were sacrificed at different time intervals following the administration (1 h, 5 h, 24 h and 48 h) under light diethyl ether anesthesia. The control rats were injected with the same volume of distilled water.

Testes and epididymides were sampled, weighed and embedded in paraffin using routine histological practice. Spermatozoa were isolated from both vasa deferentia 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 and Discussion

Hypoxia is known to compromise the fertility in man (Okumira et al., 2003) and in other mammals. It has an impact on male testicular functions such as reduced testosterone level and disturbance of spermatogenesis (Farias et al., 2008). A strong metabolic stress in spermatogenic cells is observed. Several studies provide some evidence for histopathological changes of the testis in male rats, but the observed effects could not be confidently attributed to sodium nitrite exposure (RCHAS, OEHHA, CAL/EPA, 2000).

Our histological evaluation of four-month-old old rat testis showed the presence of mature spermatozoa and their release into the tubular lumen in stage VIII of spermatogenic cycle. The spermatogenesis is organized in 14 stages according to the classification of Clermont and Perey (1957). The germ cells are arranged in five-six layers. Luminal region in all of the seminiferous tubules is clearly distinguished (Figure 1A). Besides the intact structure of seminiferous epithelium, our preliminary morphological observation of experimental rat testis demonstrated the presence of disorganized seminiferous tubules and assemblage of undifferentiated germ cells in the luminal area in some experimental animals.

Fig. 1A. Morphology of the seminiferous tubules of control rat testis. HE, x 200

Fig. 1B. Morphology of the seminiferous tubules on rat testis cross sections five hours fallowing NaNO2 application (50 mg.kg⁻¹.hw) Disorganization of seminiferous epithelium was observed. HE, x 200
There is lack of lumen in some tubules. These findings are most obvious at the fifth hour after NaNO₂ administration but may also be seen at the 24th and 48th hour (Figure 1B). Interestingly, blood vessels with larger diameter are more frequently seen compared to the control (data not shown). We did not expect alterations in seminiferous epithelium so early after sodium nitrite treatment despite its acute application. The changes are not seen in all experimental animals in group and our future work would elucidate if disorganized seminiferous tubules are due to the effect of sodium nitrite.

Literature data for the effect of sodium nitrite-induced hemic hypoxia on the male reproductive system are controversial. Male rats (Farias et al., 2005b) and rhesus monkeys (Saxena, 1995) subjected to hypobaric hypoxia showed atrophy of germinal epithelium with Sertoli cell replacing spermato- gonia as well as spermatogenic arrest at the end of the third week of exposure. No evidence for testicular pathology is identified in animals subjected to a three-day regimen of NaNO₂ injections (Bond et al., 1981).

The body reacts to hypoxia with adaptive responses, such as angiogenesis and vasodilation, thus increasing blood supply to tissues as a compensatory oxygen delivery mechanism. Local changes in testicles exposed to hypoxia include neovascularisation and an increase in temperature that would be correlated with the alterations in spermatogenesis observed in this tissue. In this respect, the response of testis to hypoxia would resemble other hyperthermia-related pathologies, such as varicocele and cryptorchidism (Farias et al., 2005b). Farias et al. (2005b) found significantly greater number and increased diameter of blood vessels in interstitial space of rats subjected to hypobaric hypoxia. Our findings about the frequently seen blood vessels with higher diameter are in agreement with these observations.

The magnitude and time dependence of testicular mass changes are very relevant for interpreting the underlying physiological changes taking place in this tissue. Our quantititative results show that the gonado-somatic index (ratio testicular weight to body weight) was elevated by 130 g.kg⁻¹ in the first hours (first and fifth hour) and returned within the normal range at later stages (Table 1). Epididymal index (ratio epididymal weight to body weight) remained at normal values although it was slightly elevated 24 hours following NaNO₂ administration (Table 1). Elevated gonado-somatic index was unexpected at an early stage after hypoxia. Literature data show changes in organs’ mass during hypoxia only if they are engaged in blood flow homeostasis (heart’ and lungs’ mass) (Hammond et al., 2001). Therefore, the elevated indices in our study could be due to increased testicular fluid volume, which may be associated with impaired hormonal balance (dysfunction in the hypothalamo-hypoph- ysal-gonadal axis), and tissue anoxia (Saxena, 1995).

We found reduction of sperm count after NaNO₂ induced hemic hypoxia. The sperm count was reduced in all experimental groups (1 h, 5 h, 24 h and 48 h) by 300 g.mg⁻¹, 470 g.mg⁻¹, 160 g.mg⁻¹ and 280 g.mg⁻¹, respectively (Figure 2). Saxena (1995) has also reported a significant decrease of semen volume in rhesus monkeys in the first week after exposure to hypoxia. Hypobaric hypoxia associated with high altitude exposure is reported to induce oligoasthenospermia with reduced motility, reduction of the total number of motile sperm and an increase of abnormal or immature spermatozoa. In animal models (rat, mouse, guinea pig, rabbit, monkey, sheep), it has been shown that hypobaric hypoxia induces partially reversible quantitative changes such as

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**Table 1**

Changes in rat gonado-somatic index (ratio of the testis weight to body weight) and epididymal index (ratio of the epididymal weight to body weight) at different time intervals following NaNO₂ administration. Data represent mean value ± SD (* p < 0.05; ** p < 0.01; *** p < 0.001)

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<thead>
<tr>
<th></th>
<th>TW/BW</th>
<th>EpiW/BW</th>
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<tbody>
<tr>
<td>Control</td>
<td>0.0055 ± 0.0006</td>
<td>0.0016 ± 0.0002</td>
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<tr>
<td>1 h</td>
<td>0.0062 ± 0.0006</td>
<td>13%, ** 0.0018 ± 0.0003</td>
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<tr>
<td>5 h</td>
<td>0.0061 ± 0.0006</td>
<td>12%, ** 0.0016 ± 0.0002</td>
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<tr>
<td>24 h</td>
<td>0.0057 ± 0.0006</td>
<td>ns 0.0018 ± 0.0003</td>
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<tr>
<td>48 h</td>
<td>0.0058 ± 0.0006</td>
<td>ns 0.0017 ± 0.0002</td>
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**Fig. 2. Changes in rat sperm count at different time intervals following NaNO₂-induced acute hemic hypoxia.**

Data represent mean value ± SD (* p < 0.05; ** p < 0.01; *** p < 0.001). Sz count – spermatozoa count
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A decrease in semen volume, sperm count and sperm motility (Gasco et al., 2005; Petrova and Ormandzhieva, 2012). Established low thyroid hormone concentrations have also been reported to affect semen quality (Chandrasekher et al., 1985).

The observed changes may be indicative of impaired spermatogenesis and suggest decreased testicular functions. Probably testis morphology, some weight indices and sperm count in mature rats are sensitive and undergo changes in the early stages after NaNO₂ acute treatment.

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