Sodium nitrite-induced hematological and hemorheological changes in rats

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Abstract
The aim of the present study is to investigate the effect of acute sodium nitrite (NaNO\textsubscript{2}) treatment on the hematological and hemorheological parameters in rats.

Mature rats were subjected to NaNO\textsubscript{2} exposure by a single intraperitoneal injection of 50 mg/kg body weight. The animals were sacrificed 1h, 5h, 24h, 48h and 5, 10, 20 days following the administration. Hematological and hemorheological parameters were measured.

Preliminary results showed significantly reduced red blood cell count (RBC), while mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were significantly increased one and five hours after NaNO\textsubscript{2} administration compared to controls. MCHC remained significant until day 20 after treatment. According to the rheological studies plasma viscosity (PV) was the highest in the controls indicating possible biochemical changes in the treated samples. The highest whole blood viscosity (WBV) was measured 10 days after injection which corresponds to the increased RBC count.

Acute treatment with NaNO\textsubscript{2} induces significant hematological and rheological changes therefore monitoring of these parameters is necessary when exposed to high doses or prolonged treatment with the compound.

Keywords: Sodium nitrite, hematological and hemorheological parameters, rats

1. Introduction

Sodium nitrite is an inorganic salt with both harmful and healthful effects \cite{16,18}. It is known as E250 in the food industry and used as a common preservative and color fixative in fish and meats. Sodium nitrite is also used as pharmacological agent in cyanide poisoning. Recent studies suggest that the vasodilator effect of NaNO\textsubscript{2} may be of therapeutic benefit in the treatment of pulmonary hypertension \cite{8}, posthemorrhagic cerebral vasospasm \cite{12}, and myocardial infarction \cite{17}. Other common uses are in fertilizers, dyes, pyrotechnics, etc. Although NaNO\textsubscript{2} can be found in drinking water, the diet is generally the most sufficient source of human exposure.

Sodium nitrite in blood is highly reactive with hemoglobin, thus affecting hematopoiesis. A major concern in considering the toxicology of NaNO\textsubscript{2} is the induction of methemoglobinemia – a condition in which there is a reduction in hemoglobin’s ability to transport oxygen.

As blood parameters are sensitive indicators of the organism’s physiological state, our study is conducted to investigate the effect of acute sodium nitrite treatment on the hematological and hemorheological parameters in rats. Based on the effects observed in rodents, it may be beneficial in predicting the risk of methemoglobinemia in humans exposed to NaNO\textsubscript{2}.

2. Methods

2.1. Experimental design
The experiments were carried out on four-month-old male Wistar rats. The animals were divided into seven sodium nitrite-treated groups (n=15 in each group) and an age-matched control group (n=15). Rats were maintained in the institute’s animal house in standard hard bottom polypropylene cages at 23°C±2°C, 12:12 h light/dark cycle and free access to laboratory chow and tap water throughout the study.

In brief, NaNO₂ was administered intraperitoneally at 50 mg/kg body weight (1 ml dosing volume). Treated animals were sacrificed at different time intervals following the administration (1h, 5h, 24h, 48h and days 5, 10, 20) under light anesthesia. The control rats were injected with the same volume of distilled water.

The animal experiments were performed in accordance with the animal protection guidelines approved by the Ethics Committee for Experimental Animal Use at IEMPAM, BAS.

2.2. Blood collection

Whole blood samples were obtained in heparinized tubes, centrifuged and plasma was stored at -20°C until further analysis.

2.2.1. Hematological parameters measurement

Erythrocyte parameters – RBC, hematocrit (Hct), mean corpuscular volume (MCV), MCH, MCHC and red blood cell distribution width (RDW) were obtained using automated hematological analyzer BC-2800 Vet (Mindray, China).

2.2.2. Rheological parameters measurement

Whole blood and plasma samples were used for measuring WBV and PV with a rotational viscometer Contraves Low Shear 30 over a wide shear rate range from 0,0237 s⁻¹ to 128,5 s⁻¹ at 37°C. All viscometric measurements were carried out within three hours after sample preparation.

2.3. Statistical analysis

Results are reported as mean values ± SD and statistically analyzed by Student’s t-test.

3. Results

Acute treatment with NaNO₂ reduced significantly RBC count 1h and 5h after exposure, while at day 10 the number of erythrocytes was significantly increased compared to controls (Fig. 1). Mean corpuscular volume (MCV) was significantly reduced in all treated groups (Fig. 2). The change in the cell volume resulted in alterations in the hemoglobin-related parameters – MCH (Fig. 3) and MCHC (Fig. 4) which were significantly increased in the experimental groups compared to the control animals. Simultaneously, acute exposure to NaNO₂ induced changes in the red blood cell distribution width (RDW) (Fig. 5). The latter parameter was reduced in all experimental groups compared to the controls with a significant difference one hour and 48h after the administration of the compound.

Hemorheological studies of whole blood samples showed decreased whole blood viscosity (WBV) after treatment with NaNO₂ (Fig. 6) except in the 48th hour and day 10 groups. The elevated WBV by day 10 following the administration of the compound corresponds to the increased RBC.

4. Discussion

Sodium nitrite is a main preservative in cured meat products, fish and some types of cheese and occurs naturally in many foods, particularly vegetables. A growing body of evidence indicates the beneficial effect of NaNO₂ therapy in some vascular diseases [4]. However, toxicity to humans and animals is well documented in nitrite overexposure [14, 16, 18]. Sodium nitrite is the major contaminant of drinking water. Its widespread use in the food industry contributes to the potential health risk if not handled cautiously.
Fig. 1. Changes in RBC count after NaNO₂ treatment. **p<0.005.

Fig. 2. Changes in MCV after NaNO₂ treatment. **p<0.005.

Fig. 3. Changes in MCH after NaNO₂ treatment. **p<0.005; ***p<0.001.
Fig. 4. Changes in MCHC after NaNO₂ treatment. ***p<0.001.

Fig. 5. Changes in RDW after NaNO₂ treatment. **p<0.005; ***p<0.001.

Fig. 6. Changes in WBV after NaNO₂ treatment.
Nitrite is present in the plasma and RBC at approximately 120 nM and 290 nM, respectively, in healthy subjects [5]. The chemical reactivity of NaNO₂ with hemoglobin may enhance heme- or iron-mediated toxicities. Nitrite is known to cause free radical generation [10], as it can stimulate oxidation of ferrous ions in oxyhemoglobin to form methemoglobin as well as various ROS [2, 6]. The nitrite ion, its metabolites, and lipid peroxidation products are supposed to react with sulfhydryl groups of the lipid bilayer and protein components of erythrocyte membrane and change its structure [11]. Nitrite-promoted Ca²⁺ influx in blood cells activates phospholipases, which increase the proportion of phospholipids with a rigid structure in the membrane [9].

The present study demonstrates the effect of acute NaNO₂ treatment on the hematological and hemorheological parameters in rats. Significantly reduced RBC count, as well as increased MCH and MCHC at the first and fifth hour following NaNO₂ administration were the major hematological findings. Oxidative damage might be a relevant cause of the initial decrease in RBC count which may be attributed to lysis or shrinkage of erythrocytes in the blood [13]. However, other investigators report that NaNO₂ administration increases methemoglobin without any effect on RBC hemolysis [3]. We have also shown that RBC count returned within the normal values at later stages and there are two mechanisms that might be responsible for that — through erythropoiesis and via the release of RBC from the hematopoietic tissues by β-adrenergic action. Other studies conducted in rats have also provided evidence for adverse effect of NaNO₂ on the hematological parameters. Similarly, a significant decrease in hemoglobin concentration, Hct and RBC count is reported [2, 7]. Moreover, white blood cell (WBC) count and lymphocyte number is shown to decrease and this is associated with the failure of the hematopoietic tissues to produce new WBC [15]. It is suggested that the decrease in RBC count, hemoglobin concentration and Hct may be attributed to microcytic and/or hypochromic anemia possibly as a consequence of the toxic effect of NaNO₂ on bone marrow, spleen and liver [1]. Our results for reduced RBC, MCV and RDW are signs for possible microcytic anemia-like changes after acute exposure to the compound.

According to the rheological studies, we measured the highest plasma viscosity in the controls indicating possible biochemical changes in the treated samples (data not shown). The highest whole blood viscosity was measured in rats 10 days after injection which corresponds to the increased RBC count. These results suggest that rheological properties of RBC might be affected by sodium nitrite-induced oxidant attack.

The above data indicate that NaNO₂ affects the hematological and hemorheological parameters in mature rats. Our future work would elucidate if NaNO₂ administration is associated with structural alterations in blood cells.

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References