FOOD PRESERVATIVES AND SPORT. SODIUM NITRITE (E250)’S INFLUENCE ON HEMATOMETRICAL PARAMETERS OF WHITE BLOOD CELLS AND PLATELETS IN RATS

(Original scientific paper)

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Abstract

The vasodilatory activity of nitrites makes them attractive for athletes in attempt to improve their performance. The aim of the study is to investigate the influence of acute NaNO2 treatment on white blood cell and platelet hematometrical parameters in experimental rat model. Male Wistar rats were injected i.p. with 50 mg/kg b.w. NaNO2. At different time periods following administration (1 h, 5 h, 24 h, 48 h; days 5, 10 and 20), the animals were sacrificed and the hematometrical parameters were obtained. Hematometrical data showed that acute in vivo NaNO2 treatment affects rat white blood cells and platelets. A statistically significant decrease of WBC and Gran one hour after the treatment and in PDW five hours after injection was obtained. A significant increase of PCT and PLT one and five hours after injection was observed. These results contribute for the elucidation of NaNO2 influence on morphological and functional properties of blood cells. Acute treatment with NaNO2 showed diverse effects on white blood cells and platelet count. The results suggest that exposure to NaNO2 would have a negative impact on immunity and blood cell aggregation.

Keywords: food preservatives, sodium nitrite, white blood cells, platelets

INTRODUCTION

Sports and the active way of life are very important factors for good health and high quality of life for the modern man. It is well known that the sport and physical activity have protective effects on cardiovascular and all-cause mortality. The analysis of Brandt & Pedersen (2010); Nocon, et al., (2008) show that concerning cardiovascular mortality, physical activity was associated with a risk reduction of 35%, whereas all-cause mortality was reduced by 33%. Recent investigations, including people with impaired glucose tolerance have found that lifestyle alterations as diet and moderate physical activity, preserves against the development of type 2 diabetes [Brandt & Pedersen (2010). The literature defines the term “a disease of physical inactivity”, which includes type 2 diabetes, cardiovascular diseases, colon cancer, breast cancer, and dementia (Pedersen (2009).

Food quality and composition are very important factors for human health and contribute to the better performance of the athletes. There are data in the literature showing that the presence of nitrates and nitrites in food is connected with an increased risk of gastrointestinal cancer and, in infants, methemoglobinemia as well (Hord, Tang & Bryan (2009). Approximately 80% of dietary nitrates are obtained via consumption of vegetables as well as fruits and processed meats. Nitrites are produced endogenously through the oxidation of nitric oxide and through a reduction of nitrate by commensal bacteria in the mouth and the gastrointestinal tract (Hord et al. (2009).

Sodium nitrite (NaNO2) is a salt and an anti-oxidant that is used to cure meats like salami, hot dogs, pepperoni, bologna, ham, bacon, SPAM etc. It blocks the growth of botulism-causing bacteria and prevents the spoilage. Sodium nitrite also gives cured meats their characteristic color and flavor (American Meat Institute). In food industry it is best known as E250. Daily sodium nitrite intake oscillates between 1.8-8.4 mg in dependence of different factors. (Hord, Tang, & Bryan, (2009) resume that consumption of red and processed meats is associated with an increased risk of certain types of cancer and chronic obstructive pulmonary disease [Norat, Bingham & Ferrari (2005); Santarelli, Pierre & Corpet (2008); Varraso, Jiang, Barr, Willett & Camargo (2007); Jiang, Paik, Hankinson& Barr, (2007); Jiang, et
METHODS

Adult male 4-month old Wistar rats (n = 49) were given a single intraperitoneal (i.p.) NaNO\textsubscript{2} injection of 50mg/kg body weight (b.w.). The treated animals were divided into seven groups according to the time of evaluation following the injection and each group included 7 animals. The control group consisted of 9 animals. At different time periods following administration (1 h, 5 h, 24 h, 48 h; days 5th, 10th and 20th), the animals were sacrificed and blood samples were prepared. Hematometrical parameters - white blood cell count (WBC), the absolute content of lymphocytes (Ly), absolute content of granulocytes (Gran), platelet count (PLT), platelet distribution width (PDW) is volume index of the heterogeneity of platelets, PCT (platelet crit) - trombokrit, the proportion (%) of whole blood occupied by platelet and mean platelet volume (MPV) were measured on automated hematological analyzer BC-2800Vet (Mindray, China). All results obtained are reported as mean values ±SD and statistically analyzed by Student’s t-test. Statistical significance was determined at p≤0.05.

RESULTS AND DISCUSSION

Blood is a two-phase suspension comprised of structure elements - red blood cells (RBCs), white blood cells (WBC) and platelets (PLT), suspended in plasma - an aqueous solution of organic molecules, proteins and salts (Baskurt & Meiselman (2003)). The whole blood rheological behavior is generally characterized by the erythrocytes – their aggregation and deformation, morphological, functional and membrane properties, as well as the interactions between blood components.

Recent studies suggest that the vasodilator effect of NaNO\textsubscript{2} may be of therapeutic benefit in the treatment of pulmonary hypertension (Hunter, et al., (2004), posthemorrhagic cerebral vasospasm (Pluta, Dejam, Grimes, Gladwin & Oldfield (2005)), and myocardial infarction (Webb, et al., (2004)). The vasodilation activity of nitrite makes it attractive for athletes in attempt to improve their performance. Nitrites are not prohibited by the doping agencies but their prolonged use raises serious concerns (Petrócz & Naughton (2010)).

It is important to know the benefits and disadvantages of excessive sodium nitrite consumption. In our previous studies we report the effects of sodium nitrite on the blood rheological properties and erythrocyte hematometric indices (Ivanov, Gluhcheva, Petrova & Antonova (2014). The aim of this study is to investigate the influence of acute (i.p.) treatment of Wistar rats with NaNO\textsubscript{2} on the white blood cell count (WBC) and platelets’ (PLT) morphological parameters.

![Fig. 1. Changes of the white blood cells after NaNO\textsubscript{2} injection. A) white blood cell count (WBC), b) lymphocyte number, c) monocytes number and d) granulocytes number. Single asterisks (*) signifies difference at p<0.05 and double asterisks (**) signifies difference at p<0.01](image-url)
The increase was possibly due to the reduced cell volume (MPV) which was significantly decreased 5 hours after 
NaNO\textsubscript{2} administration (Fig. 2b.). The increased platelet count induced a significant increase in PCT (Fig. 2a).

The nitrite had little or no effect on PDW as the values remained close the untreated control. The results suggest that exposure to NaNO\textsubscript{2} may affect PLT activity, thus altering blood coagulation (Dautov, et. al., (2014.). Our data are in agreement with those of Dautov et al. which show inhibition of PLT aggregation under hypoxic conditions induced by NO\textsubscript{2} (Dautov et al., (2014.)). According to Srihirun et al. nitrates inhibit PLT aggregation in the presence of erythrocytes, which could explain the increased number of PLT in the whole blood samples of NaNO\textsubscript{2}-treated rats.

CONCLUSION

Acute treatment with NaNO\textsubscript{2} showed diverse effects on white blood cells and platelet count. The nitrite reduced the phagocyte cell number – monocytes and granulocytes. These results suggest that the animals were susceptible to infections. The number of monocytes was not restored even 20 days after exposure to NaNO\textsubscript{2}. The results support data from Abuharfeil et al. who demonstrate the immunosuppressive effect of sodium nitrite in mice following the subchronic treatment (Abuharfeil, Sarsour & Hassuneh (2001.).

NaNO\textsubscript{2} exhibited the opposite effect on platelets (Fig.2.). Their number was significantly increased in all experimental groups except in day 20 group (Fig.2c.). The increase was possibly due to the reduced cell volume (MPV) which was significantly decreased 5 hours after NaNO\textsubscript{2} administration (Fig. 2b.). The nitrite had little or no effect on PDW as the values remained close the untreated control. The results suggest that exposure to NaNO\textsubscript{2} may affect PLT activity, thus altering blood coagulation (Dautov, et. al., (2014.). Our data are in agreement with those of Dautov et al. which show inhibition of PLT aggregation under hypoxic conditions induced by NO\textsubscript{2} (Dautov et al., (2014.)). According to Srihirun et al. nitrates inhibit PLT aggregation in the presence of erythrocytes, which could explain the increased number of PLT in the whole blood samples of NaNO\textsubscript{2}-treated rats.

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**Fig. 2. Changes of platelets' parameters after NaNO\textsubscript{2} injection. A) Plateletcrit, b) mean platelet volume (MPV), c) platelet count and d) platelet distribution width. Double asterisks (**) signifies difference at p<0.01**


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