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L-Arginine and L-glutamine as immunonutrients and modulating agents for oxidative stress and toxicity induced by sodium nitrite in rats

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ABSTRACT

Sodium nitrite ($NaNO_2$) is a flavoring, coloring and preservative agent in meat and fish products. The study aimed to evaluate the efficacy of L-arginine and L-glutamine supplementation as a potentially novel and useful strategy for the modulation of oxidative stress and toxicity induced by $NaNO_2$ in male rats. Rats were divided into six groups each of 10 rats and treated for 6 weeks: group 1 as normal control; group 2 fed standard diet containing 0.2% $NaNO_2$; group 3 and 4 fed the previous diet supplemented with 1% and 2% arginine, respectively; group 5 and 6 fed $NaNO_2$ diet supplemented with 1% and 2% glutamine, respectively. $NaNO_2$ treatment induced a significant increase in serum malondialdehyde, nitric oxide, arginase, glutathione-S-transferase activities, urea and creatinine as well as differential leucocytes%. However, a significant decrease was recorded in reduced glutathione, catalase activity, total protein, albumin and some hematological parameters as well as immunoglobulin G. On the other hand, arginine or glutamine showed a remarkable modulation of these abnormalities as indicated by reduction of malondialdehyde and improvement of the investigated antioxidant and hematological parameters. It can be concluded that arginine or glutamine supplementation may reduce oxidative stress and improve the hazard effects of $NaNO_2$.

1. Introduction

Sodium nitrite ($NaNO_2$) is present in vegetables and is routinely used as a color fixative and preservative in meats and fish (National Toxicology Program, 2001). Nitrite is recognized as a toxic compound that can induce a number of physiological disturbances when its concentration in the organism is high (Jensen, 2003). Nitrite is, however, also a natural constituent in the body, it is a potential nitric oxide (NO) donor and recent research has suggested that nitrite has important biological functions at low concentrations (Jensen, 2007).

The hazardous effect of $NaNO_2$ is derived from the reaction of nitrite with amines and amides to produce nitrosamines and nitrosamides, respectively. The toxic effects of nitrates and nitrites are well documented in mammalians, including impairment of reproductive function, hepatotoxicity and methaemoglobinemia, dysregulation of inflammatory responses and tissue injury, growth retardation and endocrine disturbance (Jahries et al., 1986).

Recent trends in controlling and treating diseases tend to favor natural antioxidant compounds rather than synthetic ones (Craig and Beck, 1999).

L-Arginine (Arg) is classified as a semi-essential or conditionally essential amino acid with numerous roles in cellular metabolism. It serves as an intermediate in Krebs–Henseleit urea cycle and as precursor for biosynthesis of protein, nitric oxide, creatine, polyamines and L-glutmate (Appleton, 2002). In some stress conditions that put an increased demand on the body for the synthesis of L-arginine, Arg becomes essential, and it is then very important to ensure adequate dietary intake of the amino acid to meet the increased physiological demands created by these situations. Conditions in which Arg becomes necessary include periods of growth and after recovery of injury. Arginine also promotes wound healing. Furthermore, arginine has several immunomodulatory effects (Lind, 2004).

L-Glutamine (Gln), traditionally considered a nonessential amino acid, now appears to be a conditionally essential during stress, injury or illness (Flaring et al., 2003). Glutamine plays an essential role, promoting and maintaining function of various organs and cells such as kidney (Conjard et al., 2002), liver (Ramos Lima et al., 2002) and pancreatic β-cells (Skelly et al., 1998). Additionally Gln plays an important role in cell proliferation, this effect has been observed in a variety of cell types including lymphocytes and enterocytes (Boza et al., 2000; Calder and Yaqoob, 1999).

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The objective of this study was therefore to investigate the role of L-arginine and L-glutamine supplementation to ameliorate the oxidative stress, hepatic and renal injury induced by sodium nitrite.

2. Materials and methods

2.1. Materials

2.1.1. Chemicals

Sodium nitrite, L-arginine and L-glutamine were purchased from El-Nasr Company, Egypt and Sigma–Aldrich, St. Louis, MO, USA, respectively. Other chemicals used were of analytical pure grade.

2.1.2. Animals

A total of 60 healthy male albino rats, weighing 95.73 ± 11.16 g were obtained from the breeding unit of the Egyptian Organization for Biological Products and Vaccines, Helwan, Egypt.

2.1.3. Diet

Standard diet was prepared following the defined composition of the AIN-93M (Reeves et al., 1993).

Composition of standard diet (g/100 g): cornstarch 62.07, casein14, sucrose 10, cellulose 5, corn oil 4, mineral mixture 3.5, vitamin mixture 1, L-cystine 0.18, choline bitartrate 0.25 and tert-butylhydroquinone 0.008.

2.2. Methods

2.2.1. Experimental design

Rats were housed in individual cages in a room maintained at 22 ± 3 °C with a 12 h light: dark cycle, and allowed free access to water and standard diet for 7 days as an adaptation period. Rats were divided into six groups of 10 rats each, and were assigned for six weeks to one of the following dietary regimens:

Group (1): rats were fed on the standard diet (control group).
Group (2): rats were fed on the standard diet containing 0.2% $NaNO_2$ (Lijinsky et al., 1983).
Group (3): rats were fed on the standard diet containing 0.2% $NaNO_2$ and 1% Arg (Ronnenberg et al., 1991).
Group (4): rats were fed on the standard diet containing 0.2% $NaNO_2$ and 2% Arg.
Group (5): rats were fed on the standard diet containing 0.2% $NaNO_2$ and 1% Gln (Shewchuk et al., 1997).
Group (6): rats were fed on the standard diet containing 0.2% $NaNO_2$ and 2% Gln.

At the end of experimental period, rats were anesthetized and scarified after 12 h food deprivation. Blood samples were collected from hepatic portal vein into two tubes, one contained EDTA for the hematological parameters determination in hemocytometer and for the biochemical parameters. The second tube contained no anticoagulant, the obtained serum was kept at −20 °C until analysis.

2.2.2. Biochemical analysis

Blood reduced glutathione (GSH) concentration was measured according to Beutler et al. (1963). Lipid peroxidation expressed as malondialdehyde (MDA) was measured in serum according to method of Draper and Hadley (1990). Serum nitric oxide (NO) and arginase activity were determined as described by Miranda et al. (2001) and Mellerup (1967), respectively. Plasma glutathione transferase (GST) and catalase (CAT) activities were estimated as previously described by Habig and Jakoby (1981) and Takahara et al. (1960), respectively.

Serum total protein, albumin, urea and creatinine were analyzed by the method of Wooton (1964), Doumas et al. (1971), Patton and Crouch (1977) and Heinegard and Tiderstrom (1973), respectively. Immunoglobulin G (IgG) was determined by using radial immunodiffusion plates according the method of Fahey and McKelvey (1965).

2.2.3. Statistical analysis

Data were analyzed by SPSS statistical package version 11.0 (Levesque, 2007). All data were means ± S.D. Data were analyzed by one-way analysis of variance ANOVA, difference between groups were determined by the post Hoc least significant difference test (L.S.D) and $P < 0.05$ was considered to be statistically significant.

3. Results

Results presented in Table 1 demonstrated that $NaNO_2$ administration for a period of six weeks resulted in a significant increase ($P < 0.05$) of serum MDA, NO levels and arginase activity, while L-arginine or L-glutamine supplementation in both concentrations significantly ameliorated these parameters.

Table 1
Effects of dietary L-arginine and L-glutamine supplementation on malondialdehyde (MDA), nitric oxide (NO) and arginase activity in sodium nitrite fed rats.

Groups		Parameters		
		MDA (nmol/ml)	NO (μmol/L)	Arginase (U/L)
Group (1)	Control	3.05 ± 0.12^{c}	6.23 ± 0.56^{e}	20.37 ± 1.44^{e}
Group (2)	$NaNO_2$	5.08 ± 0.36^{a}	29.12 ± 1.73^{a}	50.60 ± 2.79^{a}
Group (3)	$NaNO_2$ + 1% Arg	3.15 ± 0.16^{c}	15.14 ± 0.95^{c}	35.72 ± 3.23^{bc}
Group (4)	$NaNO_2$ + 2% Arg	3.86 ± 0.25^{b}	16.70 ± 1.81^{b}	31.61 ± 1.46^{d}
Group (5)	$NaNO_2$ + 1% Gln	3.13 ± 0.10^{c}	10.21 ± 1.18^{d}	36.87 ± 2.88^{b}
Group (6)	$NaNO_2$ + 2% Gln	3.21 ± 0.12^{c}	9.68 ± 1.30^{d}	33.42 ± 2.19^{cd}
L.S.D		0.21	1.34	2.45

Values are means ± SD for ten rats.
Means in the same column with different superscripts are significantly different ($P < 0.05$).

Table 2
Effects of dietary L-arginine and L-glutamine supplementation on antioxidant biomarkers in sodium nitrite fed rats.

Groups		Parameters		
		Blood GSH (mg/dl)	GST (U/L)	CAT (U/L)
Group (1)	Control	27.25 ± 2.19^{a}	36.75 ± 3.54^{f}	1374.75 ± 74.74^{a}
Group (2)	$NaNO_2$	13.38 ± 1.51^{d}	145.38 ± 12.95^{a}	753.63 ± 44.51^{e}
Group (3)	$NaNO_2$ + 1% Arg	19.50 ± 1.20^{c}	103.50 ± 8.52^{c}	1176.63 ± 42.35^{b}
Group (4)	$NaNO_2$ + 2% Arg	20.25 ± 1.39^{c}	69.13 ± 8.44^{e}	1066.38 ± 51.21^{c}
Group (5)	$NaNO_2$ + 1% Gln	25.50 ± 1.31^{b}	82.25 ± 9.36^{d}	1139.38 ± 74.60^{b}
Group (6)	$NaNO_2$ + 2% Gln	25.38 ± 1.06^{b}	124.63 ± 9.13^{b}	845.00 ± 47.66^{d}
L.S.D		1.50	9.17	57.88

Values are means ± SD for ten rats.
Means in the same column with different superscripts are significantly different ($P < 0.05$).

From the results in Table 2 it is clear that a significant decrease ($P < 0.05$) in blood glutathione content and plasma catalase activity, while a significant increase in plasma GST activity were shown in rats administrated $NaNO_2$. Arg or Gln supplementation in both concentrations induced a significant increase of GSH content and catalase activity but a significant decrease in GST activity.

The data in Table 3 shows that $NaNO_2$ induced a significant decrease of serum total protein and albumin, while a significant increase in serum levels of urea and creatinine. However, supplementation of $NaNO_2$ intoxicated rats with Arg or Gln ameliorated the nitrite adverse effects as evidenced by a significant increase of serum total protein and albumin, with a decrease of urea and creatinine levels.

Table 4 shows the effects of dietary Arg or Gln supplementation on blood picture and differential leucocytic counts as well as IgG. Analysis of variance indicated that there was a significant decrease in Hb concentration, RBCs, WBCs and lymphocytes counts as well as IgG, whereas a significant increase in monocytes and granules counts was detected in rats fed $NaNO_2$. L-arginine or L-glutamine supplementation significantly increased Hb%, RBCs, WBCs and lymphocytes counts also IgG, on the other hand significantly decreased monocytes and granules counts.

4. Discussion

Sodium nitrite and other additives are important flavoring, coloring and preserving agents in meat and fish products. However, it