EFFECTS OF BORIC ACID ON REDOX STATUS IN THE RAT LIVER

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Abstract. Boron occurs most frequently in nature as borates and boric acid (H_3BO_3), never as the free element. These compounds are toxic to all species tested at high doses, but they are not carcinogenic or mutagenic. The present work studies the in vivo influence of boric acid on the level of lipid peroxides, non-protein thiol groups and superoxide-dismutase, glutathione-peroxidase, glucose-6-phosphate dehydrogenase activities in liver tissue of rats. During an experimental period of 90 days young male Wistar rats with the same ages received a standard diet containing vitamin mix (A, E, D) and either 40 ppm boric acid (group 1) or 80 ppm boric acid (group 2). These two groups were compared to a control group treated with a standard diet containing vitamin mix. Results indicated a significant increase in lipid peroxides ($p < 0.05$) for the (group 1) and a significant decrease ($p<0.003$) for the (group 2) compared to control. Oxidative effects on G-SH metabolism were less pronounced in the (group 2) than in the (group 1). The activity of liver superoxide dismutase was unsignificantly increased. The glucose-6-phosphate dehydrogenase as well as glutathione-peroxidase activities were significantly increased for both groups of rats compared to control one.

Key words: lipid peroxides, boric acid, superoxide-dismutase, glutathione-peroxidase, glucose-6-phosphate dehydrogenase.

INTRODUCTION

The toxicity of different xenobiotics is dependent upon several factors: dose, chemical structure, interaction with other compounds and the integrity of target tissues.

The compounds resulting during the metabolism of xenobiotics can be inactive or, on the contrary, can have a high degree of toxicity. These compounds act both in the liver (main metabolic center) as well as in the other tissues. Most of xenobiotics are subjected to metabolisation, the liver being the main organ involved. Most are dealt with by a series of enzymes that have broad substrate specificity; these enzymes detoxify xenobiotics and convert them into species that are more soluble in water and thus easier to excrete.

Received February 2003.

ROMANIAN J. BIOPHYS., Vol. 12, Nos. 3–4, P. 77–82, BUCHAREST, 2002

Numerous studies have shown that boric acid and borax are absorbed from the gastrointestinal tract as well as from the respiratory tract, as indicated by increased levels of boron in the blood, tissues, or urine or by systemic toxic effects of exposed individuals or laboratory animals [12].

Boric acid is a weak acid and at physiological pH is in the form of an uncharged small molecule with a molecular volume of 71.5 Å [1], which is similar to urea (75.3 Å) and other small nonelectrolytes. Inorganic borates are not metabolized by biological systems owing to the excessive energy (523 kJ/mol) required to break the boron-oxygen bond [12]. In both human and animal studies, more than 90% of the administered dose of borate is excreted as boric acid [11].

Xenobiotics-induced oxidative stress can damage cells by multiple mechanisms, including direct oxidative damage to DNA, lipids and proteins, depletion of ATP and NAD^+, falls in GSH/GSSG ratios, increases in "free" intracellular Ca^{2+} and transition-metal ions concentrations and changes in membrane antioxidant content. For many compounds, the free radicals are directly responsible for their toxicity, while for others the free radicals generation is a later stage in the process of cell injury. Under physiological conditions, the organism uses enzymic antioxidative systems (e.g. superoxide-dismutase, catalase, glutathione-peroxidase, glutathione-S transferase) and nonenzymic antioxidative systems (e.g.: glutathione, ascorbic acid, vitamin E, etc.) in order to maintain the peroxide level within normal limits.

In this study we evaluated malondialdehyde level as expression of oxidative damage of polyunsaturated fatty acids in the liver of rats treated with boric acid, compared to controls as well as superoxide dismutase, glutathione peroxidase, glucose-6-phosphate dehydrogenase status.

MATERIAL AND METHODS

The study was performed on 60 male young Wistar rats (55 – 65 g) of the same ages and had an experimental stage of 90 days. The rats were divided into three groups, each having 20 animals: group 1– control group – received a standard diet; group 2 and group 3 received 40, respectively 80 ppm boric acid in their standard diet.

Rats were killed and liver samples trimmed and homogenized with 0.15M KCl or with 0.02M EDTA using a glass-glass homogeniser. The levels of malondialdehyde (MDA) and small-molecular weight antioxidant, GSH were immediately measured. The activities of GSH peroxidase, superoxide dismutase and glucose-6-phosphate dehydrogenase were estimated.

Lipid peroxidation products, mainly MDA, were measured spectrophotometrically after their reaction with thiobarbituric acid [4].

The concentration of non-protein thiol groups was measured after their reaction with 5,5'-dithio-bis(2-nitro-benzoic acid) at 412 nm [9]. The proteins concentration was measured using Folin's reagent at 540 nm [2].

The activity of glucose-6-phosphate dehydrogenase was measured by monitoring NADPH oxidation at 340 nm [3].

The activity of superoxide dismutase was assayed by monitoring nitroblue tetrazolium reduction as described by Spitz and Oberley [10]. The activity of cytosolic glutathione peroxidase was measured using hydrogen peroxide as substrate [7].

Results are expressed as the mean ± SD. Student's t-test at $p < 0.05$ was used for statistically significant differences between the two groups.

RESULTS

During the experimental period, no weight loss and mortality were observed, for young rats exposed to apparently subtoxic doses of boric acid. Both doses of boric acid induced lung haemolysis without liver necrosis.

The results presented in Table 1 show a significant increase in lipid peroxides ($p<0.05$) and a significant decrease ($p<0.003$) of reduced glutathione level, for the (group 1). The level of lipid peroxidation product (MDA) was decreased and reduced glutathione level was not significantly modified for the (group 2).

Table 1

MDA, G-SH and proteins level in the rat liver tissue, after 90 days of treatment with boric acid

	MDA (nmoles/g tissue)	G-SH (μmoles/mg protein)	Total liver proteins (mg protein/g wet tissue)
Control	22.13 ± 0.98	24.29 ± 2.84	295.7 ± 9.66
40 ppm boric acid	26.15 ± 1.65 $p < 0.05$	17.39 ± 4.76 $p < 0.001$	277.6 ± 12.49 $p > 0.05$
80 ppm boric acid	15.38 ± 2.05 $p < 0.003$	23.40 ± 5.66 $p > 0.05$	303 ± 7.74 $p > 0.05$

The activity of liver superoxide dismutase does not show statistically significant modifications for any doses of boric acid administered (Table 2). The liver glucose-6-phosphate dehydrogenase as well as glutathione-peroxidase activities were significantly increased for both groups of rats (Table 2).

Table 2

The activity of glutathione-peroxidase, glucose-6-phosphate-dehydrogenase and superoxide dismutase in the rat liver tissue treated with boric acid

	Glutathione-peroxidase activity (μmoles GSH oxidized / mg protein /min)	Glucose-6 phosphate dehydrogenase activity (Wroblewsky units)	Superoxide dismutase activity (SOD units/ mg protein)
Control	46.76 ± 8.09	156.3 ± 7.99	95.44 ± 5.19
40 ppm boric acid	97.21 ± 15.36 $p < 0.003$	1656.5 ± 43.3 $p < 0.001$	97.06 ± 5.25 $p > 0.05$
80 ppm boric acid	73.11 ± 11.96 $p < 0.05$	1075.6 ± 12.5 $p < 0.001$	98.3 ± 4.02 $p > 0.05$

DISCUSSIONS

Numerous studies suggest that boron interacts with other nutrients and plays a regulatory role in the metabolism of minerals, such as calcium, and subsequently bone metabolism. Although the mechanism of action has not been defined, it may be mediated by increasing the concentration of the steroid hormones such as testosterone and beta-oestradiol [5].

Two hypotheses have appeared to account for the multiple effects of boron [6, 8]. One hypothesis is that boron has a role in cell membrane function, stability, or structure, such that it influences the response to hormone action, transmembrane signaling, or transmembrane movement of regulatory cations or anions [6]. Another hypothesis is that boron is a negative regulator that influences a number of metabolic pathways by competitively inhibiting some key enzyme reactions [8]

Lipid peroxidation may lead to the production of toxic and reactive aldehyde metabolites. Among these, MDA and 4-hydroxy-2-nonenal (HNE) are the most important. The results presented in Table 1 show a correlation between variation of lipid peroxides and reduced glutathione level. These results may suggest the pro- and antioxidant effects of boron acid intake in vivo.

Glutathione is the essential cofactor for many enzymes requiring thiol-reducing equivalents, and helps keep redox-sensitive active sites on enzymes in the necessary reduced state. Glutathione status is a highly sensitive indicator of cell functionality and viability. The liver is the largest GSH reservoir. The maintenance of G-SH level in the same limit with control for the (group 2) may be correlated with decreased lipid peroxides level and increased activity of glucose-6 phosphate dehydrogenase (Table 2).

Glucose-6 phosphate dehydrogenase being a cytoplasmic enzyme, its main metabolic role is the production of NADPH in the monophosphate pathway and the defense against oxidizing agents. The results indicate a significant increase ($p<0.003$) in glucose-6 phosphate dehydrogenase activity, for both groups of rats treated with boric acid, (Table 2). NADPH generated through the monophosphate pathway is necessary for GSSG reduction and for increased steroid hormones synthesis in the presence of boric acid [5].

The liver glutathione-peroxidase activity was significantly increased for both groups of rats treated with boric acid compared to control (Table 2). We hypothesize that this raise of glutathione-peroxidase is an adaptive reaction of liver cells to the multiple effects of boron.

Boron supplementation did not increase significantly the levels of total SOD activity in the liver tissue; the decreased oxidizability of liver tissue rats treated with 80 ppm boric acid could be the result of the other mechanisms such as changes in membrane antioxidant content.

There is evidence both in vitro and in vivo systems that boric acid has affinity for hydroxyl groups; this may be the mechanism explaining the biological effects of boric acid. However, this attachment is known to be reversible and concentration dependent, responding to clearance mechanisms [12]. Through these effects, boron can affect the function or composition of several body systems, including blood, brain, and skeleton, in a positive manner, which demonstrates that boron is a beneficial element, if not an essential mineral element, at physiological amounts [8].

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