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BORON AS A METABOLIC INTERCEPTOR IN LIVER DISEASES: BIOCHEMICAL ROLE AND NUTRITIONAL SIGNIFICANCE

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ABSTRACT: Mineral elements such as copper, iron, manganese, and zinc are regarded as trace elements essential for life as they generally occur in trace amounts (mcg/g tissue) and evidences for their essentiality are substantial and non-controversial. Boron can also be added to the list of essential trace elements, as evidence is more than circumstantial for its essentiality; boron deficiency has been shown to interrupt the life cycle of some higher animals. Findings have suggested that boron at physiological and pharmacological amounts can promote health and reduce risks leading to disease. In the present manuscript, we review the results describing boron as a protector in acute form of liver injury. The effect of boron was studied on mild and moderate forms of liver injury induced by administering thioacetamide to Wistar strain of rats. Boron was administered as borax (4 mg/kg) orally for three consecutive days followed by thioacetamide administration, and the rats were sacrificed six hours after administering the last dose of thioacetamide. Serum marker enzymes of hepatic injury, and oxidative stress markers were analyzed. The results suggest that the liver injury can be modulated and attenuated by boron treatment at physiological concentrations, and counteracts the oxidative stress. Boron probably acts as a metabolic interceptor of some biochemical pathways involved in maintaining the oxidant/antioxidant balance in the tissue. Nutritional significance of boron is also discussed in the paper.

Introduction

An ever-expanding number of mineral elements have received attention as being of possible importance in the prevention of disease with nutritional roots or for the enhancement of health and longevity. Some of these elements such as copper, iron, manganese and zinc are essential and are required by humans in amounts of milligrams per day. These elements are classified as essential trace elements, as these elements generally occur in the body in mcg/g of tissue concentration. The evidence for the essentiality of these elements in humans is substantial and non-controversial, and specific biochemical functions have been defined for all of them. Other elements such as boron and chromium are being investigated for their essentiality, and reports are available showing that at physiological and pharmacological amounts these elements can promote health and reduce risks leading to disease. In the present manuscript, we summarize the results of a study where boron was found to minimize the changes caused by thioacetamide, a hepatotoxic agent that inflicts liver damage of varying degrees depending upon its dose and treatment schedule.

Boron has long been known as an essential trace element for higher plants (Warrington, 1923), and has often being recognized as being beneficial to animals and human (Nielsen, 2000). Food and drink of plant origin, especially noncitrus fruits, leafy vegetables, nuts, pulses, legumes, wine and cider are among more prominent sources of boron. Boron is rapidly absorbed and excreted mainly as undissociated $B(OH)_3$ in urine. It is more likely that most ingested boron is converted to $B(OH)_3$, the dominant inorganic boron species at gastrointestinal tract pH. Boron is distributed throughout soft tissues and body fluids at concentrations mostly between 0.015 and 0.6 mcg/g fresh tissue (Abou-Shakra et al., 1989). The mechanism of its transport in the body has not been defined. Findings have suggested the health promoting and disease risk reducing effect of boron at physiological and pharmacological amounts. In the present paper, we describe boron as protector of liver damage in experimental animal model of liver necrosis and fulminant hepatic failure (FHF). Role of boron as a metabolic interceptor of altered biochemical pathway in disease state is discussed, and the importance of boron as a nutrient is emphasized in the present paper.

Materials and methods

Adult rats (Wistar strain) were used in the study after obtaining ethical clearance. Animals were kept in an environmentally controlled room with a 12-hour light and 12-hour dark cycle. They received standard pellet

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diet and water ad libitum. All chemicals used in the study were of analytical grade and procured from local market, except thioacetamide that was purchased from Sigma Chem. Co. (USA).

Administering a single dose of 400 mg/kg has been reported to induce liver necrosis, while repeated injections for three consecutive days lead to massive necrosis, inflammation, centrivenous congestion and hydroponic changes that result in acute liver failure. In case of hepatic failure model, supportive therapy by subcutaneous administration of 5% dextrose (25 mL/kg b.wt.) and 0.9% sodium chloride with potassium (20 mEq/L) was given every 12 hr to avoid weight loss, hypoglycemia, and renal failure. Boron was administered to rats orally as borax (4 mg/kg) for three consecutive days every 24 hr followed by the administration of thioacetamide, which was given an hour after the last dose of borax was administered. In mild necrosis model, borax was given for a similar duration of time. Control rats were treated with intraperitoneal injection of a similar volume of normal saline. It was ensured that all the animals (treated or untreated) are sacrificed at the same time and all the biochemical estimations are completed on the same day. Following completion of the treatment protocol, blood was collected by cardiac puncture, and animals were sacrificed by cervical dislocation. Serum markers of liver injury such as aminotransferases (alanine aminotransferase and aspartate aminotransferase) and alkaline phosphatase were estimated using diagnostic kits supplied by Span Diagnostics (India). Xanthine oxidase was assayed using the postmitochondrial supernatant to ascertain the source of reactive oxygen species. Malondialdehyde and glutathione were measured from the whole tissue lysate. The method used for these estimations have been described earlier (Pawa, Ali, 2004). Protein content was determined in each biological sample by Lowry's method using bovine serum albumin as reference standard.

Statistical analysis

All the data were expressed as Mean ± S.E. of six rats. Values obtained in each experimental group were compared to the group of rats with FHF using Student's t-test. The level of statistical significance was chosen as $*p < 0.05$.

Results and discussion

Mineral elements present in trace amounts (mcg/g tissue) in animal tissues have received attention as being of possible importance in the prevention of disease with nutritional roots, or for the enhancement of health and longevity. Trace and ultra trace elements essential for living organisms play various roles such as working in concert with proteins/organic coenzymes, participating in oxidation-reduction reactions, imparting stability and three dimensional structure to important biological molecules, and controlling important biological processes such as inhibiting the enzymatic reactions, and facilitating the binding of the molecules to receptor sites on cell membranes. They also have been reported to alter the structure or ionic nature of membranes to prevent or allow specific molecules to enter a cell, and induce gene expression. Mineral elements such as copper, iron, manganese, and zinc are regarded as trace elements essential for life, and evidences for their essentiality are substantial and non-controversial. Boron can also be added to the list of essential trace elements, as evidence is more than circumstantial for its essentiality (Nielsen, 2002).

Several studies have employed thioacetamide to produce a model of liver necrosis and acute liver failure in experimental animals (Poniachik et al., 2002; Chu et al., 2001; Ali et al., 2001). The models produced by thioacetamide in rats have been validated, and have proved reliable and satisfactory. Thioacetamide administration causes significant increase in the level of serum marker enzymes associated with liver damage and other biochemical parameters. As can be seen in Table-I and Figures provided below, treatment with thioacetamide resulted in varying degrees of damage to liver. Activity levels of enzymes associated with the liver damage increased significantly after thioacetamide treatment (Table 1, and Fig. 1). In the experimental animals receiving boron for three consecutive days, a significant decrease in the elevated levels of the biochemical markers associated with liver injury can be observed.

Malondialdehyde and the tripeptide glutathione (γ-L-Glutamyl-L-cysteinylglycine) are two important biochemical indicators of oxidative damage. Earlier studies have revealed an increase in tissue malondialdehyde content, and a concomitant decrease in the level of hepatic glutathione following thioacetamide administration. The results presented in Figure 2, and 3 are in agreement with the previous findings, and suggest an increase in hepatic lipid peroxidation in thioacetmide treated rats. A

Table 1. Activity levels of aminotransferases measured from the serum obtained from animals with necrosis and acute liver failure without and with boron treatment

Group	sALT (U/ml)		sAST (U/ml)	
	Untreated	Boron treated	Untreated	Boron treated
Control	33.5 ± 3.20	31.8 ± 4.63	132.8 ± 7.08	131.1 ± 4.74
Necrosis	110.7 ± 6.51	50.2 ± 5.24	179.7 ± 5.78	148.0 ± 4.22
ALF	2,600 ± 38.5	1810 ± 32.65	4,400 ± 50.6	3,070 ± 48.0

sALT: serum alanine aminotransferase or glutamate pyruvate transaminase (GPT); sAST: serum aspartate aminotransferase (formerly, glutamate oxaloacetic transaminase, GOT); ALF: acute liver failure

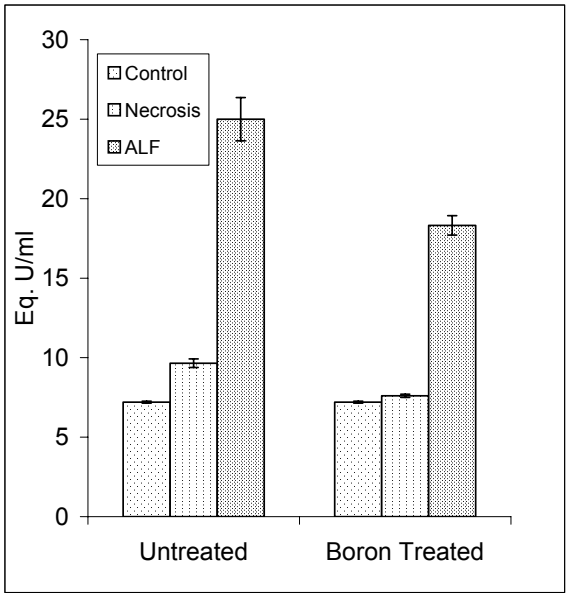

Figure 1. Effect of boron treatment on serum alkaline phosphatase level in liver necrosis and fulminant hepatic failure in rat model. ALF: acute liver failure

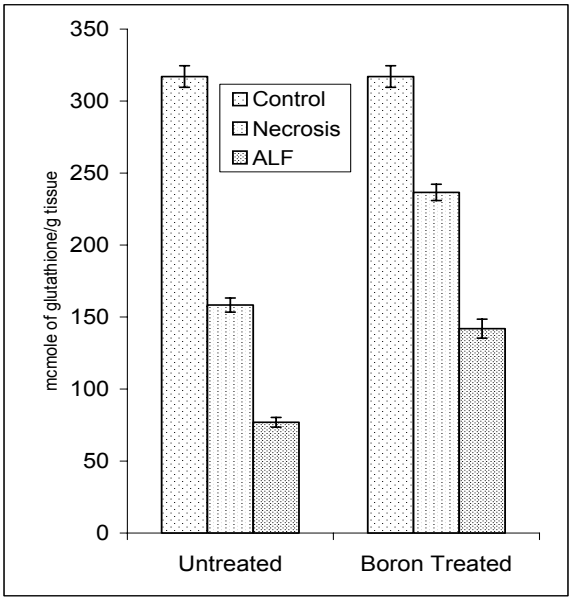

Figure 3. Effect of boron treatment on the level of glutathione in liver tissue of rats with liver necrosis and fulminant hepatic failure

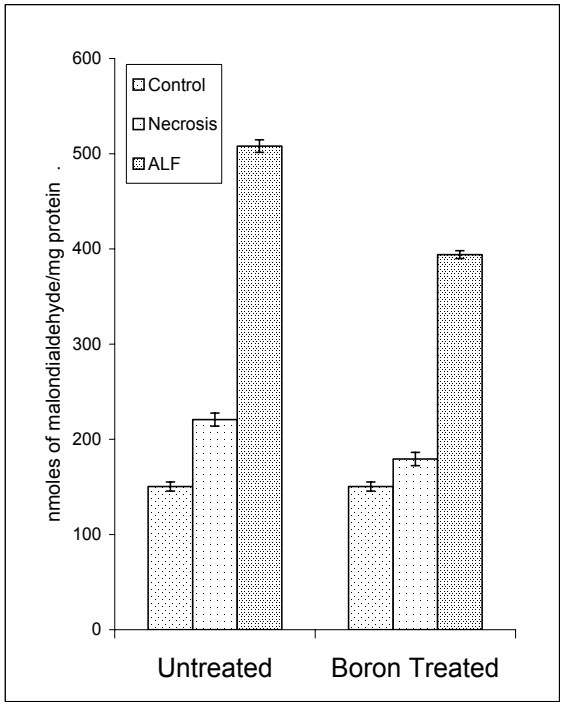

Figure 2. Effect of boron treatment on hepatic lipid peroxidation in rat liver necrosis and fulminant hepatic failure

concomitant decrease in the level of hepatic glutathione in mild and massive liver necrosis can be seen (Fig. 3). The results indicate that boron treatment could bring down the changes associated with the oxidative stress produced as a result of injury to the liver. Decreased lipid peroxidation is an indicator of the free radical/peroxide mediated damage to the tissue, and elevated glutathione suggests the tissue response to oxidative injury.

Hepatic injury is inflicted by a variety of mediators. Role of reactive oxygen moieties such as superoxide anion radical, hydroxyl radical and peroxides is well known in various forms of liver injuries. When liver cells are exposed to excess ROS, oxidative stress occurs and affects many cellular functions by various mechanisms such as the alteration in gene expression through activation of transcription factor, or induction of permeability transition in mitochondria with fatal consequences. Increased lipid peroxidation is a manifestation of the tissue damage produced due to production of free radicals in excess or due to the depletion in the level of endogenous antioxidants such as tripeptide glutathione.

Reactive oxygen species have been reported generated in various forms of liver injury from various sources. Xanthine oxidase, a molybdoflavoprotein, has been recognized as an endogenous source of reactive oxygen metabolite. In a study from our laboratory (Ali et al., 2001), partially reduced oxygen moieties produced as by-product during the reaction catalyzed by xanthine oxidase, have been shown to inflict damage to hepatic tissue. The increase in hepatic lipid peroxidation following mild and moderate necrosis in rat model used in this study has been shown suggesting the role of reactive oxygen moieties. We explored xanthine oxidase as a source of the oxidant moieties, and observed an increase in the activity level of xanthine oxidase (Fig. 4). Boron treatment could bring down the elevated xanthine oxidase activity level, thereby reducing the generation of oxidant moieties. This could explain the lesser degree of damage to hepatic tissue following boron treatment even when the damage was massive.

Trace elements such as Zn, and Se have long been recognized as protectors of tissue damage that act either by their antioxidant properties or their possible interaction with other trace elements in maintaining the cellular

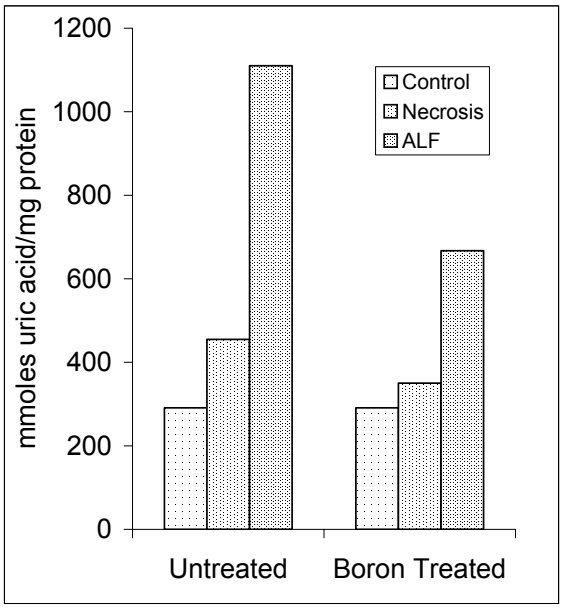

Figure 4. Effect of boron treatment on the activity level of xanthine oxidase measured in rat liver necrosis and FHF

harmony (Zhou et al., 2002; Goel, 2000; Al-Bader et al, 1997; Cabre et al., 2001; Whanger, 1992). Boron is a ubiquitous element widely distributed in nature in the form of borates at low concentrations in soils and rocks. Boron is released from these minerals by the natural weathering processes in the form of boric acid, which is water soluble and biologically available. Being a dynamic trace element, boron has been observed to exert multiple effects. Together, all the results presented and discussed in the paper suggest that boron in the form of borax can alleviate the damage to hepatic tissue, and that the mechanism of action of boron in preventing the damage may involve some pathway related to oxidative stress. Borax is most important and commonly found compound of boron. It is completely absorbed when taken by the oral route of exposure (Murray, 1998). No specific biochemical function has been assigned to boron, even for plants for which boron has been known since 1923 to be essential and for which boron deficiency has a multiplicity of effects. In biological materials, boron exists mainly bound to oxygen, and its biochemistry is essentially that of boric acid; dilute aqueous boric acid solutions comprise $B(OH)_3$ and $B(OH)_4$- species at the pH of blood (Woods, 1994). Boric acid forms ester complexes with hydroxyl groups of organic compounds, preferably when the hydroxyl groups are adjacent and cis (Zittle, 1951). Formation of complexes of compounds such as pyridoxine, riboflavin, dehydroascorbic acid, adenosine-5-phosphate, and pyridine nucleotides may be biologically important because, in vitro, it results in the competitive inhibition of enzymes such as oxidoreductases that require cis-hydroxyl-containing pyridine or flavin nucleotides as cofactors. A couple of hypotheses are provided here to explain the hepatoprotective effect of boron. Boron can either act as a metabolic regulator (inhibiting some key enzyme reactions) or may act indirectly as a proton donor, exerting a particular influence on the cell membrane structure and function. Boron has affinity for cis-hydroxyl groups present in the membrane phospho-inositides and glycolipids (Benderour et al., 2000). Boric acid can form ester complexes with the adjacent and cis-hydroxyl groups of organic compounds. The importance of the proper hydroxyl arrangement is demonstrated by the fact that polysaccharides made of carbohydrates such as glucose, glucuronic acid and xylose do not react with borate because they do not have the required paired hydroxyl groups. Hydroxyl groups distorted from a single plane also do not react well with borate. Pyridine nucleotides, adenosine 5-phosphate, riboflavin, pyridoxine and dehydroascorbic acid are among the many biological substances that complex with boron. This may be biologically important as in vitro these complexes can result in competitive inhibition of some enzymes such as oxidoreductases, which require cis-hydroxyl containing pyridine or flavin nucleotides as cofactors. As discussed earlier, xanthine oxidase-derived ROS appear to play an important role in the pathogenesis of hepatic injury. At physiological concentrations and pH, boron may react with one N group or one to four hydroxyl groups on specific biological ligands like pyridine (e.g., NAD^+ or $NADP^+$) or flavin (FAD) nucleotides and serine proteases. In this manner, boron inhibits pyridine nucleotide requiring oxidoreductase like xanthine dehydrogenase. However, other mechanism operating simultaneously such as elevated cAMP level may not be denied. Compounds of boron are known to interfere with oxidative phosphorylation processes of mitochondria, elevate cAMP levels, and inhibit enzymatic hydrolytic activities (Hall et al., 1980). Increase in cAMP supposedly stabilizes the lysosomal membranes and thus boron might inhibit liver necrosis.

Nutritional significance of boron

Knowledge about the role of boron in nutrition and also its clinical aspects is just emerging, and indicators of boron status are still being established. Although the element boron has no defined biochemical function in human, it apparently has a role that influences the metabolism and utilization of several other nutrients. Thus, described deficiency signs and the pathological consequences of inadequate boron may be numerous and variable. A plasma boron concentration below 25ng/mL might be indicative of a low boron status (Nielsen, 2002). A powerful homeostatic regulation, and the consumption of diets with different types of foods from different sources may help maintain the content of boron in cells/tissues and body fluids. Food and drink of plant origin, especially noncitrus fruits, leafy vegetables, nuts, pulses, legumes, wine and cider are among more prominent sources of boron, and their adequate consumption might have beneficial effects.

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