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Research Paper

Body Weight Reducing Effect of Oral Boric Acid Intake

Erhan Aysan[1]✉, Fikrettin Sahin[2], Dilek Telci[2], Mehmet Emir Yalvac[2], Sinem Hocaoglu Emre[3], Cetin Karaca[4], Mahmut Muslumanoglu[1]

1. Department of General Surgery, Faculty of Medicine, Bezmialem Vakif University, Turkey
2. Department of Bioengineering, Yeditepe University, Turkey
3. Department of Biochemistry, Istanbul Educational and Research Hospital, Turkey
4. Istanbul University Animal Research Laboratory, Turkey

✉ Corresponding author: Erhan Aysan, Address: Bezmialem Vakif University, Vatan Caddesi, Fatih, Istanbul, Turkey. Tel: +902124531700; Fax: +902125310092; e-mail: dr@webcerrah.com

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Abstract

Background: Boric acid is widely used in biology, but its body weight reducing effect is not researched.

Methods: Twenty mice were divided into two equal groups. Control group mice drank standard tap water, but study group mice drank 0.28mg/250ml boric acid added tap water over five days. Total body weight changes, major organ histopathology, blood biochemistry, urine and feces analyses were compared.

Results: Study group mice lost body weight mean 28.1% but in control group no weight loss and also weight gained mean 0.09% ($p<0.001$). Total drinking water and urine outputs were not statistically different. Cholesterol, LDL, AST, ALT, LDH, amylase and urobilinogen levels were statistically significantly high in the study group. Other variables were not statistically different. No histopathologic differences were detected in evaluations of all resected major organs.

Conclusion: Low dose oral boric acid intake cause serious body weight reduction. Blood and urine analyses support high glucose, lipid and middle protein catabolisms, but the mechanism is unclear.

Key words: Boric acid, oral intake, body weight

Introduction

Boron is a very stable ametal and in nature it is found as borates. Borates are chemical compounds which contain oxoanions of boron. The natural borate content of groundwater and surface water is usually small. Concentrations of boron in groundwater throughout the world range widely, from 0.3 to 100 mg/litre. Concentrations of boron in drinking water have wide ranges, depending on the source, but for most of the world the range is estimated to be between 0.1 and 0.3 mg/litre. The richest sources of boron are fruits, vegetables, pulses, legumes, and nuts. Dairy products, fish, meats, and most grains are poor sources of boron. The mean daily intake of boron in the diet is estimated to be near 1.2 mg/day (1).

Boron is immediately to the left of carbon atom in the periodic table. It is very similar to carbon atoms, so many carbon-based molecules are the same as the boron-based molecules. The most important difference between boron and carbon is its smaller atomic structure. For that reason boron has been used for about 30 years in boron neutron capture therapy (2). Carbon creates chain- or ring-shaped molecules, but boron creates very different forms -- cages, clusters, etc (3).

The essential role of the trace element boron in plants has long been established (4).

Boron is an essential nutrient for animals (5). Boron is used in a wide range of products, including glass, detergents, fire retardants, fibers to reinforce plane fuselages and body armor, and in superhard materials (3).

In this research we aimed to investigate the effects of oral boric acid intake on body weight. We hypothesized that low dose oral boric acid intake causes seriously weight loss without any side effects.

Methods

This study was performed at the Experimental Animal Production and Research Laboratory of Cerrahpasa Medical School, Istanbul University and was approved by the appropriate Animals Ethics Committee. All protocols were in accordance with the regulations governing the care and use of laboratory animals of the declaration of Helsinki.

Mice were divided into two groups, with 10 mice in each group. Eight weeks old twelve bal-c out-bred female mice were used. The animals were kept in standard metabolic cages specifically for mice from which daily urine and feces were detectible. A 12-hour light/12-hour dark cycle was used for illumination of the room where mice were placed.

Boric acid intake of mice was provided by adding it to drinking water. 0.28mg boric acid (2mg Bor Atac DF®, %14 Boric Acid, TMT Co, Tekirdag, Turkey) added 250ml tap water and melted with 3 min. agitation.

In day 0, mice were put in metabolic cages after weighing. Per days they were weighed ante meridiem and data recorded. In five days, mice were fed with standard pellet feed manufactured specifically for small animals and drank boric acid added tap water. No any eat or drink changing or limitation was used.

After five days total urine and feces uptakes recorded and animals were sacrificed with cervical dislocation. Anterior midline abdomino-thoracal incision was performed and for analyses of blood serum parameters cardiac puncture was performed with 21 gauge sterile injector. After the aspiration major abdominal organs; liver, spleen, stomach, intestine, colon, and thoracic organs; thyroid, thymus and with cranial incision brain were resected.

Premier evaluation parameter of this study is total body weight changes of mice. Secondary evaluation parameters were; histopathologic changes of major organs, changes of blood biochemistry, total urine volume, urine uroblinogen level, feces volume and gall acids in feces.

Morphological Evaluation: After resection, all organs were fixed in formula. After dehydration, they were embedded in paraffin. Cross sections of 5 mm were prepared, stained with hematoxylin and eosin, and evaluated by light microscopy at a magnification of x100. All evaluations were performed by a pathologist who was blinded to methods and groups.

Statistical Evaluation: Statistical analyses were performed using GraphPad Prisma V.3 software. Results were evaluated with a confidence interval of 95% and $p<0.05$ level. In addition to descriptive statistical methods (mean, standard deviation, median), the Mann Whitney U (MWU) test for inter-group comparisons, the Wilcoxon test for comparison of in-group variables.

Results

Amount of main daily drinking water values of the groups was not statistically different between the groups (3.18ml in control group and 3.50ml in study group, $p= 0.433$, $z= -0.784$). In calculation, mice in the study group received approximately 0.2mg/kg/day of boric acid.

Body weight changes of the groups and statistical analyses are revealed in Table 1. Study group mice lost body weight at a mean 28.1% in five days but in control group no weight loss; their weight gain averaged 0.09%. Body weight changes in study group were statistically more significant than control group ($p<0.001$). Day 0, day 5 body weight differences were not statistically significant in control group but significant in study group ($p=0.005$).

Total drinking water and urine outputs were not statistically different in the groups (table 1). Cholesterol, LDL, AST, ALT, LDH, amylase and urobilinogen levels were statistically significant high in study group. Other variables were not statistically different (table 1).

No histopathologic differences were detected in evaluations of all resected organs; liver, spleen, stomach, intestine, colon, thyroid, thymus and brain.

Discussion

Boron is widely used in medicine. Boron supplementation in rats' diets enhances liver glycogen deposition (6) and in chicks' diets increases shear fracture energy of the bones (7, 8). Boron supplementation of a semipurified diet for weaning pigs improves feed efficiency and alters plasma lipid metabolites (9). Boron influences the serine proteases, where boron forms a tetrahedral adduct with the active site of serine (10). In human study of Nielsen et al., supplemental boron increased serum testosterone and estradiol concentrations in postmenopausal women

(11). Because of the atomic structural similarity boron is an alternative to carbon in drug design. Boromycin, an antibiotic compound produced by streptomyces. The first clinically tested boron-based drug is Velcade® (bortezomib, Millennium Pharm., USA) which is used to treat multiple myeloma (12).

The acting range of boron is very wide but physiological and metabolic mechanisms are not clear. An important role of boron is metabolic regulation, because it complexes with a variety of substrate or reactant compounds in which there are hydroxyl groups in favorable positions (13).

The oxidoreductase enzymes are competitively inhibited in vitro by borate or its derivatives which require pyridine or flavin nucleotides, are inhibited as borate competes for NAD, or flavin co factor. Some examples are aldehyde dehydrogenase (14), xanthine oxidase (15), and cytochrome b5 reductase (16). Borate apparently complexes with the ribosyl cishydroxy groups of NAD (17). Key pathways of energy substrate metabolism contain members of oxidoreductase enzymes that are inhibited by boron. There is considerable evidence that physiologic amounts of dietary boron modulate both energy substrate utilization and mineral metabolism (15-17).

Table 1: Body weight changing and variables of the groups with statistical analyses

	Control Group			Study Group				
	Mean	Median	Std. Dev.	Mean	Median	Std. Dev.	z	p
Day 0 Body Weight (g)	21,10	21,00	1,31	20,60	20,00	2,51	-,956	0,339
Day 5 Body Weight (g)	21,30	21,00	1,16	14,80	14,75	1,30	-3,797	<0,001
Body Weight Difference (g)	0,20	0,00	0,95	-5,80	-5,75	1,74	-3,792	<0,001
Drinking water (ml/5 days)	16,90	17,00	2,08	17,50	17,00	1,51	-,784	0,433
Creatinin (mg/dl)	0,16	0,15	0,07	0,15	0,15	0,05	-,213	0,831
Cholesterol (mg/dl)	78,30	78,00	16,63	151,30	142,00	27,93	-3,781	**<0,001**
Trigliserid (mg/dl)	80,50	81,50	17,28	94,50	82,00	27,70	-,946	0,344
HDL (mg/dl)	31,50	30,50	10,00	53,20	51,00	9,90	-3,187	**0,001**
LDL (mg/dl)	15,20	16,00	4,78	81,00	75,00	22,54	-3,784	**<0,001**
Albumine (g/dl)	3,56	3,50	0,35	3,63	3,60	0,31	-,582	0,561
AST (U/l)	331,30	281,00	203,40	739,70	717,00	244,44	-3,099	**0,002**
ALT (U/l)	44,60	40,00	20,47	81,60	66,00	46,72	-2,649	**0,008**
Na (mmol/l)	140,80	141,50	2,82	141,30	141,00	3,13	-,420	0,675
K (mmol/l)	4,24	4,30	0,62	4,16	4,15	0,56	-,152	0,879
Ca (mg/dl)	8,73	8,55	0,46	8,79	8,90	0,39	-,343	0,732
LDH (U/l)	973,90	996,50	467,67	2362,20	2309,00	405,14	-3,704	**<0,001**
CK	4433,40	4526,50	1427,82	5648,50	5914,00	1827,28	-1,663	0,096
CKMB	6059,30	6385,00	1124,87	6138,60	6373,00	1393,36	-,227	0,821
Amilaz (U/l)	2678,70	2345,50	756,16	3437,90	3272,00	593,17	-2,192	**0,028**
Lipaz (U/l)	59,30	60,00	15,87	59,00	65,00	13,52	-,152	0,879
Total bilirubine (mg/dl)	0,00	0,00	0,00	0,00	0,00	0,00	,000	1,000
Direct bilirubine (mg/dl)	0,00	0,00	0,00	0,00	0,00	0,00	,000	1,000
Total protein (g/dl)	5,94	6,00	0,23	5,90	5,80	0,37	-,660	0,509
Fecal Bile Acids (mg)	2,20	2,00	0,42	2,10	2,00	0,32	-,610	0,542
Urobilinogen (mg/dl)	2,00	2,00	0,82	3,60	4,00	0,52	-3,466	**0,001**
Urine Volume (ml/5 days)	9,30	9,50	1,06	8,90	9,00	1,20	-,783	0,434

Blevins et al. revealed that boron may alter metabolism by regulating activation of manganese enzymes and/or ametal activated enzymes. They have shown that boron inhibits certain enzymes, e.g., 6-phosphogluconate dehdrogenase and aldolase, by binding to substrates or enzyme substrate complexes in plants (18). Lots of studies have shown that supplemental boron stimulates proton pumping in plants, causes hyperpolarization of the membrane potential, and increases potassium uptake. Along with these findings, there have been reports of a boron-mediated increase in ferricyanide reduction (19-22). There is also strong evidence that boron is involved in lignin biosynthesis and in cell wall cross-linking in plants (23). Barr and Morre et al hypothesized that the effects of boron on membrane function may be associated more directly with proton pumping or changes in a redox system in the plasamalemma (24, 25).

Although suggested amount of boron is 3mg/kg diet for animals (26) but daily boron intake is changed in wide ranges and toxic doses are very high. Toxicity studies focused on short-term and long-term exposure results of boric acid. In a 13-week study, mice were fed diets containing boric acid at approximately 0, 34, 70, 141, 281, or 563 mg of boron per kg of body weight per day. At the two highest doses, increased mortality and degeneration or atrophy of the seminiferous tubules was observed. In all dose groups, extramedullary haematopoiesis of the spleen of minimal to mild severity was seen (27). In the same study, mice received approximately 0, 275, or 550 mg of boric acid per kg of body weight per day in the diet in two years. This research demonstrated that increased mortality rates and non-neoplastic testicular lesions in males. In the 2-year study, the dogs received the boric acid in the diet 0, 1.5, 2.9, or 8.8mg per kg of body weight per day. An additional group received 29mg of boron per kg of body weight per day for 38 weeks. Testicular atrophy was observed in two test dogs receiving borax at 26 weeks and in the two and one dogs, respectively, killed after 26 or 38 weeks of boric acid consumption. (28).

The mutagenic activity of boric acid was examined in mouse, hamster, mouse embryo cells, and human fibroblasts but revealed that boric acid does not cause any gene mutations (29, 30). Tumor incidence was not enhanced in studies in which B6C3F1 mice received 0, 2500, or 5000 mg of boric acid per kg of feed for 103 weeks (27) and Sprague-Dawley rats received diets containing 0, 117, 350, or 1170 mg of boron per kg of feed for 2 years (28).

In this research, we revealed that low-dose (0.2mg/kg) oral boric acid intake cause seriously body weight reducing in five days and applied Turkish Patent Institude (date: May,27,2011; no: 2011/05210). In literature research, we did not find any data about the effects of boric acid on body weight reduce except Willig and Curtze's research in 1952. But we could not reach the full text and/or abstract of this article (31). Oppositely some animal researches revealed that over physiologic amounts (3mg/kg/day) of dietary boron supplementation causes weight gaining (10). Some toxicity researches expressing body weight reducing in the high boric acid intake doses. In the Lee et al. study with rats at concentrations of 0, 500, 1000, or 2000 mg of boron per kg of feed for 30 or 60 days, reported that body weights were not consistently affected (32). Weir et al. revealed that received 88 mg boron per kg of body weight per day reduced rats' body weights (28). A toxicology study done by the US Department of Health and Human Services demonstrated that 275 and 550 mg of boric acid per kg of body weight per day intake mice reduced body weights 10–17% after 32 weeks (27).

Our study reveals that very low dose (0.2mg/kg) oral boric acid intake ensures serious body weight reduction in mice. To express this dramatic body weight loss (28.1%) in a short time period we hypothesized firstly that boric acid had a diuretic effect. We collected urine output but reached no statistical differences compared with the control group. Secondarily, we analyzed blood biochemistry, urine, feces and evaluated major organ histopathology. We reached that only blood cholesterol, LDL, AST, ALT, LDH, amylase and urine urobilinogen levels were statistically high in the study group.

It is clear that in the animal and human biology energy sources is sequencing by glucose, lipid and protein expenditure. We hypothesized that high amylase (glucose elimination enzyme), cholesterol and LDL levels are results of high glucose and lipid catabolism. According to this opinion, -as a protein elimination enzyme- CK values were high in the study group but not statistically different in the control group level. The result of high catabolic metabolism, high levels of AST, ALT and urobilinogen as hepatic function markers supports this hypothesis.

In summary, this research shows that low dose oral boric acid intake cause seriously body weight reducing. Our blood and urine analyses results are supporting high glucose, lipid and middle protein catabolisms, but the mechanism is unclear.

Maintenance of obesity treatment and success of weight loss is highly variable and include some of serious questions (33). Adipose tissue and its glucose and lipid related metabolism is the target area. Insulin

sensitivity and other indexes of insulin secretion or action have been associated with changes in body weight or composition (34,35). Various aspects of glucose and lipid metabolism have been linked to individual differences in energy balance over time. The most widely studied is insulin sensitivity (33-36). Life time of adipocytes is other important point for obesity treatment (37,38). Inducing apoptosis of adipocytes may be most effective way to weight loss.

There are some questions that have not found answers. Effects of different doses of boric acid intake on body weight must be evaluated. Additionally the effects of low dose oral boric acid intake on different kind of laboratory animals (such as rats, rabbits) must be evaluated, especially the mechanism of action. For this reason we are planning a full complement of boric acid dose as well as other kinds of studies on animal. We will perform cell proliferation assay and real time PCR analyses on adipose tissue derived mesenchymal stem cells for evaluation of molecular changes in the adipose tissue level.

Conflict of Interest

The authors have declared that no conflict of interest exists.

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