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Neuroprotective effects of boric acid against fluoride toxicity on rat synaptosomes

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Abstract

Objective: Fluoride toxicity primarily contributes to the production of reactive oxygen and nitrogen derivatives, trigger the cell death pathways by causing lipid peroxidation and DNA damage. Boric acid (BA) contributes to preservation of membrane integrity and function and maintenance of redox balance due to its high affinity to some metabolites in the organism. The aim of this study was to investigate the protective effect of BA on neurodegenerative processes against the toxic effects of sodium fluoride (NaF) administered at different doses on rat brain synaptosomes.

Material and Methods: Synaptosomes obtained from the rat frontal cortex were administered at different doses of sodium fluoride (NaF) to determine the most toxic dose of NaF. Determined toxic dose of NaF for synaptosomes and BA concentrations were administered in vitro at 37°C for 30min and then the parameters of malondialdehyde (MDA) level, superoxide dismutase (SOD) activity, Na/K ATPase activity and DNA fragmentation value were measured spectrophotometrically.

Results: There was a statistically significant difference between measured parameters, when the 80mg/L NaF group was compared with the control group. We found that 10 and 25 mM BA treatment provided a significant improvement in MDA, SOD, Na/K ATPase and DNA fragmentation compared to the 80mg/L NaF group. The 5 mM BA concentration was not found effective dose according to other doses.

Conclusion: In conclusion, BA has potential for neuroprotective effects against cellular damage caused by NaF. The results suggest that the BA can be a neuroprotective therapeutic agent for fluoride toxicity.

Key words: Sodium fluoride, Synaptosomes, Boric acid, Neuroprotection.

Introduction

Fluoride (F), chemically ionic element, can produce free oxygen and nitrogen radicals (ROS and RNS, respectively) by affecting the antioxidant metabolism (1). Due to the electronegative structure of F, which means that it is negatively charged and tends to form fluorine ions, it can pass through cell membranes via ion channels (2). Excessive F uptake causes fluorosis, an important health problem, which is characterized by defects in skeletal and tooth structure (3). The main cause of fluorosis is contaminated drinking water with organic and inorganic wastes. Since F in drinking water has an ionic structure, it is absorbed rapidly through the intestinal epithelium and interferes with metabolic processes by accumulating in the different organs of the biological systems (4). In vivo studies have found that F added to drinking water of rats causes toxic effects and accumulates in soft tissues such as lung, liver, heart, brain and kidney (5).

Furthermore, F-induced ROS production reduces glutathione (GSH) levels as well as inhibition of antioxidant enzymes such as catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx) (6). Increasing lipid peroxidation is also an important biomarker of oxidative stress. In vivo studies have been shown that F exposure enhances lipid peroxidation due to increased ROS production in rat brain tissues (7). Besides, F exposure has been shown to cause genotoxic effects with chromosome anomalies and DNA damage (8).

Synaptosomes as a prototype of nerve tissue can fulfill many different metabolic functions. They are widely used owing to high mitochondrial contents, easy preparation and demonstration of synaptic functions (9). Synaptosomes have provided valuable information about the molecular mechanisms underlying neurotransmitter release, aging,

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and the pathogenesis of neurodegenerative diseases, and thus become a useful tool for monitoring molecular and bioenergetic changes in synapses (10). Synaptosomes are highly vulnerable to oxidative stress due to high unsaturated fatty acid content and high oxygen consumption such as brain (11).

Boric acid (BA) is a compound found in living organisms as a trace element. The boric acid, which is a common form of boron, is soluble in water and can incorporate into biochemical processes. BA, a monobasic molecule, contains hydroxyl groups in the chemical structure and releases protons during the reaction (12). Owing to high affinity of BA to some important molecules involved in biochemical and physiological processes such as nicotinamide adenine dinucleotide, flavin adenine dinucleotide, glycolipids, glycoproteins, and oxidoreductases may play an important role in cell membrane integrity and redox metabolism. BA is used in a lot of fields from industry to agriculture. When products containing BA are consumed, it rapidly crosses the bloodstream through the gastrointestinal tract (13). Recent studies have provided evidence that BA can be used in the treatment of some types of cancer (14). Previous studies have reported that BA has protective effects against inflammatory and oxidative damage (15). BA is involved in hormone metabolism, transmembrane signaling, and various enzymatic systems and acts as an antioxidant (16,17).

In this study, we aimed to investigate the neuroprotective effects of BA against F toxicity on account of the increase in the prevalence of studies on fluorosis in recent years. To verify our hypothesis, malondialdehyde (MDA) levels, SOD activities, DNA fragmentation and Na/K ATPase activities were measured to reveal the neuroprotective effects of BA following sodium fluoride (NaF) exposure of rat brain synaptosomes.

Material and Methods

Animals and Experimental Design

Eight healthy male Wistar albino rats (weighing 250±50 g) were supplied by Medical and Surgical Experimental Animal Applications and Research Center, Eskisehir. Experimental procedures were carried out according to the decision of Experimental Animals Ethics Committee of Eskisehir Osmangazi University (Approval number: 650). The rats were maintained under controlled conditions at 22°C ± 5°C and 45% ± 5% relative humidity with 12-hour periods (dark / light). Anesthesia was performed by intra-muscular injection at 45±5 mg/kg ketamine + 10±2 mg/kg xylazine doses, and then the unconscious rats were decapitated. Rats' frontal cortex was removed and divided into 4 equal cuts and the cuts were stored at -80°C until the day of the experiment.

In this study we investigated the neuroprotective effects of BA at 5, 10 and 25 mM concentrations versus the toxicity caused by NaF. We first researched which of the 20, 40 and 80 mg/L NaF doses were more toxic. We then administered different doses of BA treatments after determining the most toxic dose of NaF on the synaptosomes.

Preparation of synaptosomal fractions

In this study, crude synaptosomal fractions were prepared according to the modified method of Whittaker et al (18). Brain cuts from previously healthy rats were distributed randomly to experimental groups as 6 cuts in each group (n=6). The cuts were homogenized on ice with 10 mM 4-(2-Hydroxyethyl) piperazine-1-ethanesulfonic acid (HEPES) and 30 µM sucrose. The homogenates were first centrifuged at 3000xg for 10 minutes at 4°C and then the supernatants were taken and centrifuged once more at 15000xg for 20 min at 4°C. The remaining pellets were re-suspended in saline and rat brain synaptosomal fractions were obtained. According to the determined experimental groups, synaptosomes were exposed to at 20, 40 and 80 mg/L NaF and 5, 10 and 25 mM BA (Sigma, B6768, Germany) concentrations for 30 minutes at 37°C. Synaptosomal protein levels were measured according to the biuret method (19).

This method is used to demonstrate the presence of peptide bonds in the samples. The reaction of Cu+2 with the two peptide bonds is based on the principle of purple color formation, and the reaction product chelate product is measured spectrophotometrically at 540 nm.

Measurement of synaptosomal malondialdehyde (MDA) levels

The quantitative determination of lipid peroxidation is based on the color reaction between MDA and thiobarbituric acid (TBA). Synaptosomal MDA levels were measured at 532 nm according to the method reported by Ohkawa et al (20). In short, 0.6 ml rat synaptosomal fraction was added to sample 4 ml of sodium dodecyl sulphate (8%; Merck, 817034 Germany), and then 2 ml of acetic acid (% 0.6, pH 6.5; Merck, 100063, Germany) and 2 ml thiobarbituric acid solution (% 20, pH 4; Merck, 108180, Germany) was added to the reaction medium. The final concentration was adjusted to be 5 ml and heated in a water bath at 100°C for 60 minutes. After this process, it was centrifuged at 4000 rpm for 10 minutes and then spectrophotometric measurement was performed. The results were expressed as nmol/mg protein.

Measurement of synaptosomal superoxide dismutase (SOD) activities

SOD activity in liver tissue was measured according to the method of Sun et al (21). Briefly, the determination of SOD activity is based on the inhibition of nitro blue tetrazolium (NBT, Sigma, 74032, Germany) reduction of super oxide anion resulting from reaction of xanthine with xanthine oxidase. The reaction was started by adding 50 µl of xanthine oxidase to the reaction medium. Superoxide dismutase activity was measured spectrophotometrically at 560 nm for 5 min. One unit SOD was defined as the enzyme amount causing 50% inhibition of NBT reduction. The result were indicated as Unit/mg protein.

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Measurement of synaptosomal Na/K ATPase activities

Na/K ATPase activities were initiated by adding 5 µl of synaptosomal fraction to the reaction medium. Subsequently, nicotinamide adenine dinucleotide (Sigma, N1636, Germany) oxidation was measured at 340 nm for 10 minutes at intervals of 30 seconds (22). The data were indicated as U/mg protein.

DNA fragmentation values

DNA fragmentation was performed spectrophotometrically at 660 nm by reaction of the synaptosomal fractions with diphenylamine, and the data were indicated as a ratio of pellet to supernatant (23).

Statistical analyzes

Data obtained from experimental studies were evaluated using SPSS 21.0 Windows program. One-way ANOVA test was used to determine whether the results were statistically significant (P <0.05). Post hoc Tukey HSD test was used for comparison among the experimental groups.

Results

As shown in Figure 1, NaF exposure caused an increase in lipid peroxidation on synaptosomes, thus increasing MDA levels. MDA levels of 80 mg/L NaF group were significantly higher than control group (P <0.01). 5 mM and 10 mM BA concentrations treatment provided an amelioration by reducing effect at MDA levels, while 25 mM BA concentration treatment group was almost obtained similar results to the control group (P <0.001). In addition, we can say that the increase in MDA levels against fluoride toxicity showed a dose-dependent decrease with BA treatment.

NaF exposure was found to cause a significant decrease in SOD and Na/K ATPase activities on synaptosomes compared to the control group (Figure 2 and 3). BA treatment provided protective effect against NaF toxicity and increased SOD and Na/K ATPase activities. 25 mM BA concentration among all doses resulted in the most improvement in SOD and Na/K ATPase activities against 80 mg/L NaF toxicity group (P <0.001). However, 5 mM BA concentration did not provide a significant difference in SOD and Na/K ATPase activities compared with the 80 mg/L NaF group (P >0.05).

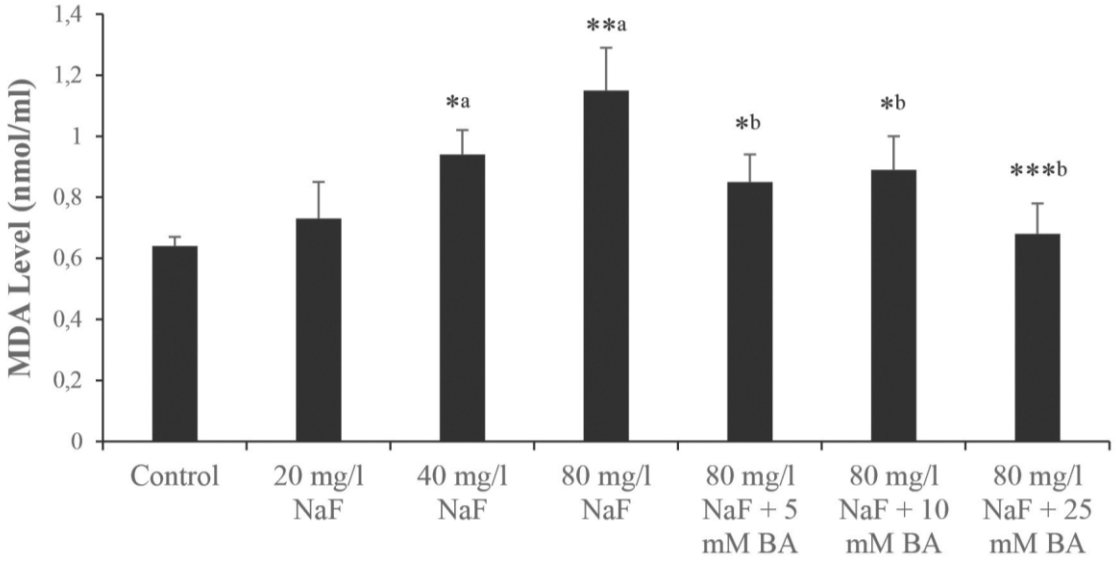

Figure 1: The neuroprotective effects of BA on MDA levels against NaF-induced toxicity on rat brain synaptosomes. All data are expressed as mean ± SEM (n=6 in each group). a: As compared to control group. b: As compared to 80 mg/L NaF group. *P <0.05, **P <0.01, ***P <0.001. NaF: Sodium fluoride. MDA: Malondialdehyde. BA: Boric Acid.

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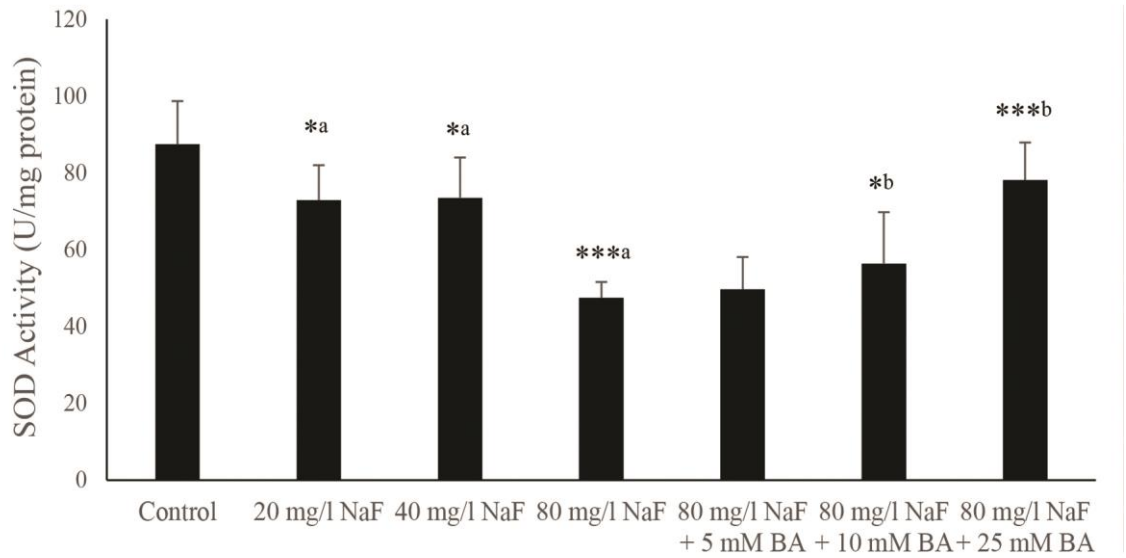

Figure 2: The neuroprotective effects of BA on SOD activity against NaF-induced toxicity on rat brain synaptosomes. All data are expressed as mean ± SEM (n=6 in each group). a: As compared to control group. b: As compared to 80 mg/L NaF group. * P <0.05, *** P <0.001. NaF: Sodium fluoride. SOD: Superoxid dismutase. BA: Boric Acid.

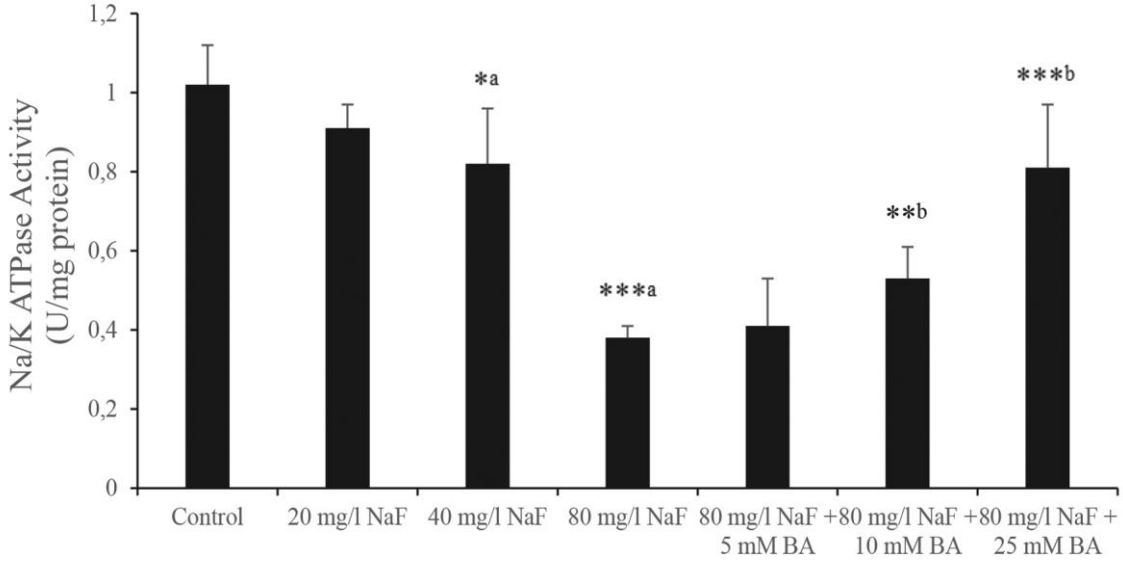

Figure 3: The neuroprotective effects of BA on Na/K activity against NaF-induced toxicity on rat brain synaptosomes. All data are expressed as mean ± SEM (n=6 in each group). a: As compared to control group. b: As compared to 80 mg/L NaF group. * P <0.05, ** P <0.01, *** P <0.001. NaF: Sodium fluoride. BA: Boric Acid.

The 10 and 25 mM BA concentrations treatment resulted in a statistically significant reduction in DNA fragmentation value compared with the 80 mg/L NaF group (P <0.01). On the other hand, 80 mg/L NaF+5 mM BA group did not cause a statistically significant decrease in DNA fragmentation (P>0.05).

As shown in Figure 4, the highest decrease/improvement in DNA fragmentation levels after NaF exposure was obtained at 25 mM BA concentration (P <0.001).

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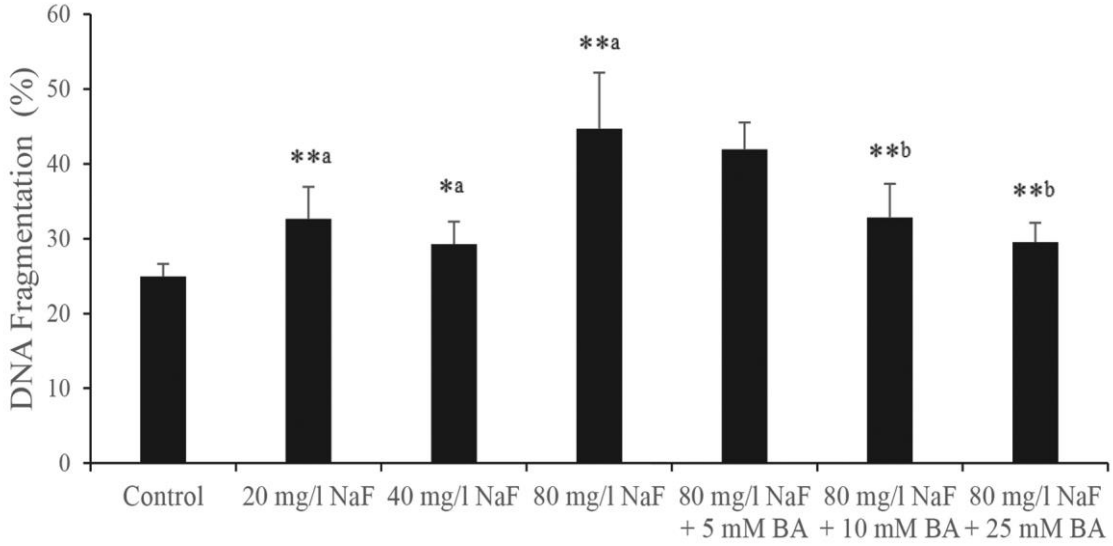

Figure 4: The neuroprotective effects of BA on DNA fragmentation against NaF-induced toxicity on rat brain synaptosomes. All data are expressed as mean ± SEM (n=6 in each group). a: As compared to control group. b: As compared to 80 mg/L NaF group. * P <0.05, ** P <0.01. NaF: Sodium fluoride. BA: Boric Acid.

Discussion

In this study, neurotoxic effect of F on rat brain synaptosomal fractions and also the evolution of neuroprotective effects of BA were investigated. Especially, 80 mg/L NaF exposure were found to cause cellular damage by triggering oxidative stress. It has been determined that the antioxidant capacity also reduce due to increased reactive oxygen species by F exposure in synaptosomes. Since the F chemical structure is highly electronegative, in vitro and in vivo studies have been shown to cause oxidative stress-induced cellular damage by up-regulation of reactive oxygen species (24,25).

Oxidative stress is associated with neurotoxicity of unsaturated long chain fatty acids occurring in mitochondrial dysfunction and neurodegenerative diseases (26). Synapses that play an important role in neuronal signal transduction are highly correlated with oxidative stress-induced neurotoxicity. Synaptosomes are highly vulnerable to lipid and protein oxidation due to their high mitochondrial content and energy consumption in presynaptic neuronal axons.

Therefore, we examined the neuroprotective effects of BA, an important antioxidant, against NaF toxicity. Our results consistent with the literature, we found that NaF exposure increased MDA levels in synaptosomes by increasing oxidative stress (27,28).

Also we found that NaF-induced increased MDA levels showed a reduction with BA treatment. This suggests that BA acts as a potential antioxidant against lipid peroxidation.

SOD, a component of the antioxidant mechanism, plays an essential role in protecting cellular integrity against peroxidative damage resulting from ROS (29). İn vivo studies have been showed that BA contributed to antioxidant mechanism by providing upregulation of SOD against increased oxidative stress (30). BA treatment on rat brain synaptosomes has significantly improved antioxidant enzyme levels due to its free radical scavenger effects. In other words, BA helps protect cellular integrity by supporting antioxidant defense system. Similar to our results, decreased SOD activity due to increased oxidative stress showed an increase with BA treatment (31).

Na/K ATPase is a membrane protein that plays an important role in maintaining the electrochemical membrane potential in cells. It is also involved in the provision of intracellular and extracellular electrolyte balances (32). F binds to the proteins of ion channels in cell membranes and inhibits them, causing the deterioration of membrane potential (33). F exposure has been reported to inhibit Na/K ATPase activity in brain tissue (34). In our study, Na/K ATPase activities in synaptosomal fractions were reduced after NaF exposure, and then BA treatment was improved in Na/K ATPase activities.

F exposure has been found to cause chromosomal abnormalities by increasing the frequency of micronucleus and gene mutations in cell lines (35). Previous studies have reported that oxidative stress, DNA damage, activation of apoptotic pathways and cell cycle changes were induced by fluoride in rats (36,37).

Zhang et al. (38) reported that 80 mg/l fluoride exposure in rat hippocampal neurons showed a positive correlation between ROS formation and DNA damage. Our data obviously suggested that F exhibits genotoxicity by increasing DNA fragmentation. We can infer that 10 and 25 mM BA treatment resulted in a significant reduction in DNA fragmentation rate by preventing oxidative stress.

Conclusion

High concentrations of F exposure have been shown to cause severe oxidative stress-induced neurodegeneration. Experimental studies on the detection of neurodegenerative damage caused by fluorosis are increasing day by day. In this study, we found that BA has neuroprotective effects against cellular damage caused by fluoride. BA is taking significant steps towards becoming a new therapeutic agent, especially by giving positive results on neurodegenerative diseases. This fundamental study of the possible neuroprotective effect of boric acid against fluoride neurotoxicity will provide new perspectives for both researchers and clinicians to work towards the therapeutic use of boric acid. But there is needed more meticulously designed molecular studies regarding boric acid as an important protective agent against oxidative stress.

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Ethical issues: All Authors declare originality and ethical approval of research. Responsibilities of research, responsibilities against local ethics commission are under the Authors responsibilities. The study was conducted under defined rules by the Local Ethics Commission guidelines and audits.

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