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Environmental boron exposure does not induce DNA damage in lymphocytes and buccal cells of females
DNA damage in lymphocytes and buccal cells of boron exposed females

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ABSTRACT

Boron (B) compounds are essential for plants and animals and beneficial for humans in nutritional amounts. I animals and humans increasing evidence have shown beneficial effects on B compounds on nutrition and on antioxidant status. The genotoxic effects of environmental B exposure in women living in boron-rich and boron-poor areas was examined in this study. For this purpose, the DNA damage in the lymphocytes and buccal cells of females were assessed by Comet and micronucleus (MN) assays respectively. No significant difference was observed in the DNA damage of the lymphocytes of B exposed groups of female volunteers in Comet assay. Even buccal micronucleus (MN) frequency observed in the high exposure group was significantly lower than the low exposure group ($p < 0.05$). The results of this study came to the same conclusions of the previous studies that boron does not induce DNA damage even under extreme exposure conditions.

1. Introduction

Boric acid (BA) and borax are inorganic boron (B) compounds which have been widely used in many manufacturing processes. They have been suggested to be essential for plants and animals and beneficial for humans in nutritional amounts [1,2]. Increasing evidence have shown the nutritional benefits and antioxidant properties of B compounds in animals and humans [3]. It is suggested to be essential for the maintenance of bone functions possibly affecting the absorption of vitamin D, magnesium and calcium [4]. It is reported that supplements of 3 mg of B/day increased the 17-β-estradiol and testosterone levels in post-menopausal women [5,6]. They have been reported to play an important role in biological systems although the exact biochemical functions of B compounds have not been yet fully identified. Regulations in the cell membrane functions and enzyme activities in oxidative metabolism are the most proposed mechanisms [3,7].

South Marmara Region of Turkey is the location of B industry. Because of the geological matters, B concentrations in drinking water may be very high in residential areas close to mining areas and in some vicinity of Bigadic. B concentrations between 8.50 and 29.00 mg/L have been reported in the drinking water. People living in that area have been exposed to high amounts of B for many years *via* drinking water [8–10]. Public concern about the unfavorable effects of high level of B exposure in the people living in such residential areas has gained attention [11].

Although negative results in a large number of mutagenicity assays in animals and in *in vitro* culture systems indicate that B compounds especially BA and borax are not genotoxic, there are very few data on the genotoxicity of B compounds in humans.

The present study is an extension of our B project which was published earlier by our study group [9,10,12] and has been conducted to investigate the genotoxic effects of environmental B exposure in women

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living in boron-rich and boron-poor areas. For this purpose, the DNA damage in the lymphocytes and buccal cells of females were assessed by Comet and micronucleus (MN) assays respectively.

2. Material and methods

This study was approved by the Ethics committee of the Ankara University School of Medicine (Ethical Approve Number: 20-853-14, dated 08/12/2014). All participants gave their informed written consent prior to participation in the project. The participating females have been living in this area since they were born.

2.1. Description of the sampling areas and study groups

The study was performed in two districts of Balıkesir; Bandırma and Bigadic, which are located in the Marmara Region of Turkey. Bandırma, away from the boron deposits and mining areas, is the production and exportation zone for the produced BA and some borates. Female volunteers (n = 92) were sampled from Bandırma where the environmental B exposure is negligible. Bigadic has the largest B deposits in Turkey which compose colemanite ($2CaO{\cdot}3B_2O_3{\cdot}5H_2O$) and ulexite ($Na_2O{\cdot}2CaO{\cdot}5B_2O_3{\cdot}16H_2O$). Boron deposits in Bigadic are located around small villages of Iskele and Osmanca. The “Bigadic Boron Works” which is the largest employment area in this region is located in Osmanca village. In total, 107 female volunteers from Bigadic, residing in the villages of Iskele and Osmanca, voluntarily participated to our study. B concentrations in drinking water sources were periodically measured in the study areas nearly for 20 years [8,13,14]. Boron concentration in some drinking sources of Iskele was even much higher (18.04 mg B/L) than the limits reported in drinking water guidelines of European Union (EU) (1 mg B/L) [15] and World Health Organization (WHO) (2.4 mg B/L) [16]. Therefore, environmental exposure was the major route of B exposure for the female volunteers of Bigadic. Detailed information about the study areas was given in our former study [17].

Volunteers participating in the study are grouped according to their blood boron concentrations as follows:

Low exposure group (n = 143): blood B concentrations lower than 100 ng B/g blood

Medium exposure group (n = 29): blood B concentrations between 100–150 ng B/g blood

High exposure group (n = 50): blood B concentrations higher than 150 ng B/g blood

Low exposure group consisted females from mostly Bandırma whereas medium and high exposure groups were females from Bigadic.

2.2. Questionnaire

In order to get an information on some possible confounders (alcohol, smoking, pesticide application) and some demographic data a detailed questionnaire from the volunteer were obtained. After completing the questionnaire, blood and buccal cell samples were taken. Since as pregnancy monitoring was not the aim of the study, pregnant women are not included.

2.3. Sampling and preparation of biological samples

2.3.1. Sampling and preparation of blood samples

Blood samples were collected from female volunteers in a heparinized blood tube (˜5 mL). Blood samples were transferred to Leucosep® isolation tubes without loss of freshness, and lymphocytes were isolated from blood samples [18] and diluted to 10,000 cells in mL for the Comet test.

2.3.2. Sampling and preparation of buccal cell samples

The buccal cell samples were taken by gently rubbing the inside of the right and left cheeks with a wet tongue depressor after the volunteers rinsed their mouths with water.

2.4. Boron analysis

Boron analysis in blood was carried out as previously described [17]. Briefly, sfter digestion of blood samples (Mars 6, CEM, Germany), B was quantified by an established and validated ICP-MS (Agilent 8800 ICP-QQQ, Agilent Technologies, Germany) method in the “no gas” mode. To verify accuracy of the measurements reference serum (Seronorm™ Trace Elements Whole Blood L-1) was carried along each batch of samples. For quality assurance of the measurement, calibration blanks and recalibration check points were determined periodically every 25 samples.

2.5. Comet assay

The Comet assay was performed in lymphocyte samples [19]. After lymphocyte isolation, the cells were embedded on agarose gel, lysed and fragmented DNA strands drawn out by electrophoresis to form a comet. After electrophoresis, the slides were neutralized and then incubated in 50, 75 and 98% of alcohol for 5 min each. The dried microscopic slides were stained with 60 µl ethidium bromide (EtBr) and scored with a fluorescence microscope (Leica® DM 100) under green light. A single scorer randomly selected and captured 100 cells using the Perceptive Instruments COMET Assay IV analysis system at 40× magnification. Tail % intensity was selected as the image analysis parameter.

2.6. Micronucleus assay in buccal cells

The buccal micronucleus (MN) cytome assay has been used to measure the DNA damage (micronuclei and/or elimination of nuclear material by budding buds), cytokinetic defects (binucleated cells) and proliferative potential (basal cell frequency) and/or cell death (condensed chromatin (CC), karyorrhectic (KHC), pyknotic (PYC) and karyolitic (KYL) cells). The slides were fixed in 80% methanol and air-dried within the same day of sample collection. The MN assay in buccal cells was performed as previously described [20,21]. For each volunteer, 2000 buccal cells (1.000 from each of the duplicate slides) were scored by light microscopy and cells frequencies were expressed as per thousand.

2.7. Statistical analysis

All results were presented using mean, standard deviation and minimum-maximum values and statistics were made using the "IBM SPSS Statistics, version 23.0" program. All data were tested for normal distribution according to the Komolgorov-Smirnow normality test. The significance of the difference between the mean values for the data with normal distribution was checked by ANOVA or t test. If ANOVA test results were found to be statistically significant, "Tukey HSD" test was applied from Post Hoc tests following ANOVA test. Correlation between normal distribution data made by Pearson correlation test and the correlation between non-normal distribution data was also made by Spearman rank test. The $\chi 2$ test was used to check the significance of the differences between the categorical variables. P values of less than 0.05 were considered as statistically significant.

3. Results

3.1. Comet assay

No significant difference was observed in the DNA damage of the lymphocytes of B exposed groups of female volunteers ($p > 0.05$) (Table 1).

Table 1
Comet test results in the lymphocytes according to blood boron concentrations of all female volunteers.[a]

	Low Exposure Group (n = 143) < 100 ng B/g blood	Medium Exposure Group (n = 29) 100-150 ng B/g blood	High Exposure Group (n = 27) > 150 ng B/g blood
% Tail Intensity	5.58 ± 3.83 (0.62–16.51)	5.26 ± 2.61 (2.12–13.47)	5.80 ± 2.96 (2.10–15.75)

[a] Mean ± Standard deviation (minimum-maximum).

Table 2
Micronucleus frequencies in the buccal cells according to blood boron concentrations of all female volunteers.[b]

	Low Exposure Group (n = 143) < 100 ng B/g blood	Medium Exposure Group (n = 29) 100-150 ng B/g blood	High Exposure Group (n = 27) > 150 ng B/g blood
Micronucleus Frequencies	8.12 ± 6.81[a] (0–28)	6.27 ± 5.58 (0 – 19)	5.73 ± 6.30[a] (0–27)

[a] Statistically difference between groups.
[b] Mean ± Standard deviation (minimum-maximum).

3.2. Micronucleus assay in buccal cells

Although buccal MN frequency observed in the high exposure group was significantly lower than the low exposure group ($p < 0.05$), no significant difference was found in the MN frequencies between the medium exposure groups and the other groups (Table 2). On the other hand, when evaluating the abnormal cells (binucleated (BN), condensed chromatin (CC), karyorrhectic (KHC), karyolitic (KYL), pyknotic (PYC), nuclear bud (NBUD)) other than MN in buccal mucosa cells, no statistically significant difference between the groups was found in the BN, KHC and KYL results. CC frequencies of medium exposure group was significantly lower than low exposure group. PYC and NBUD frequencies of medium and high exposure groups were found significantly lower when compared to low exposure group ($p < 0.05$) (Table 3).

4. Discussion and conclusion

Boron is found abundantly in nature, though in compounds and in combination with sodium and oxygen. Since man inorganic B compounds are commercially important, the number of scientific studies on human safety aspects and biological effects of B compounds is increasing.

It was reported that BA and borax do not have mutagenic effects in bacterial and animal tests [22]. Negative results in a large number mutagenicity assays in *in vitro* test systems and in animals indicate that B compounds are not genotoxic [23–25]. In the human blood cultures, B compounds at different concentrations of 2.5, 5 and 10 μM did not show genotoxic effects as they did not increase the frequencies of sister chromatid exchanges (SCEs), MN rates and chromosomal aberrations (CAs) in the peripheral lymphocytes but they have been shown to protect against vanadium and titanium dioxide induced DNA damage *in vitro* [23,25].

Table 3
Abnormal cell frequencies other than MN in buccal cells.[a]

	Bandırma + Bigadic (n = 199)		
	Low Exposure Group (n = 143) < 100 ng B/g blood	Medium Exposure Group (n = 29) 100-150 ng B/g blood	High Exposure Group (n = 27) > 150 ng B/g blood
BN	13.24 ± 5.50 (0–26)	12.93 ± 7.20 (1–30)	10.46 ± 4.21 (3–19)
CC	24.56 ± 20.89[a] (0–121)	13.54 ± 17.25[a] (1–77)	19.19 ± 21.84 (0–96)
KHC	3.39 ± 4.37 (0–25)	2.68 ± 3.71 (0–12)	2.85 ± 3.60 (0–12)
KYL	11.36 ± 13.60 (0–89)	17.32 ± 41.97 (1–226)	10.23 ± 13.54 (0–70)
PYC	8.00 ± 8.33[a] (0–36)	4.64 ± 5.53[a] (0–17)	4.92 ± 7.90[a] (0–38)
NBUD	1.07 ± 1.27[a] (0–7)	0.54 ± 0.92[a] (0–3)	0.73 ± 1.37[a] (0–6)

LEG, low exposure group; MEG, medium exposure group; HEG; high exposure group; BN, biucleated; CC, condanse chromatin; KHC, karyorrhectic; KYL, karyolytic; PYC, pyknotic; NBUD, nuclear bud.
[a] Mean ± Standard deviation (minimum-maximum).

Recent findings show that B compounds even in the highest concentrations of 500 mg/L do not have genotoxic effects in human blood lymphocytes [24] but they exert antigenotoxic properties against several toxic chemicals including heavy metals such as cadmium (Cd), arsenic (As), lead (Pb) and mercury (Hg) [3,25,26]. BA reduced the formation of DNA double strand breaks caused by cytotoxic chemotherapeutic agents such as irinotecan, etoposide, doxorubicin and hydrogen peroxide (H_2O_2) in human epithelial cell cultures [27].

Boric acid significantly reduced the H_2O_2 induced oxidative DNA damage in Chinese hamster lung fibroblast cells (V79). The protective effects of BA against oxidative damage in V79 cells at 54, 108, 540, 1080 and 2160 ng/ml B equivalents concentrations were proven in the *in vitro* study [28].

In animals, there are also very few studies indicating no genotoxicity of boron compounds. The DNA damage as assessed by Comet assay was found to decrease in rats receiving supra-nutritional amounts of B at the doses of 100 mg/kg in rats [29].

Turkez et al. evaluated the protective role of borax on aluminum induced genotoxicity in rats, using liver MN assay as the indicator of genotoxicity. No significant increases in micronucleated hepatocytes (MNHEPs) in borax treated rats at the doses of 3.25 and 13 mg/kg bw were observed but simultenous treatment with borax significantly decreased the genotoxic effects of aluminum chloride ($AlCl_3$) in rats [30].

In our study with 199 women living in Bandırma and Bigadic districts, where the former is the area distant from B deposits and mining areas whereas the latter is the area close to B deposits, no significant DNA damage was observed in the lymphocytes of the medium and high B exposure group compared to the low exposure group. This is the first study conducted with large numbers of females assessed by Comet assay. And in our study, no significant difference was found in the MN frequencies between the medium exposure groups and the other groups yet buccal MN frequency observed in the high B exposure group was significantly lower than the low exposure group ($p < 0,05$). ın our medium and high exposure groups, the frequencies of some abnormal cells such as CC, PYC NBUD were even lower than the low exposure group. Korkmaz et al. also evaluated MN frequency in exfoliated buccal cells of total 60 women consisting 30 women from B-rich and 30 women from B-poor areas. They also found that MN frequencies did not differ significantly [31].

In the previous Chinese and Turkish studies done in highly B exposed populations, no significant increases were observed in the DNA damage in the sperm samples of workers from B mining areas. The mean blood B concentrations of exposed workers in the Chinese and Turkish study were reported as 499.2 ± 790.6 (20.4–3568.9) ppb and 223.9 ± 69.5 (152.8–454.0) ng B/g blood, respectively [9,32].

The present study in females living in Bandırma and Bigadic was performed between 2014 and 2017 as stated in our previous data [17]. The periodically monitored B concentrations in drinking water have not shown major changes in local B concentrations with time. In these females also no B-mediated unwanted effects on spontaneous and induced abortion, stillbirth, infant and neonatal death, congenital abnormalities, birth weight of newborns were observed.

In the sperm samples of workers under the exposure conditions of boric acid production plant in Bandırma, the protective effects of B exposure have even been reported regarding the sperm morphology, sperm motility and DNA integrity [12].

In conclusion, the results of our study also indicate that high levels of B do not induce genotoxic effects in females since no significant increases in MN frequencies in the buccal cells and DNA damage in the lymphocytes were observed.

Conflict of interest

The authors declared that they have no conflict of interest.

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