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ORIGINAL RESEARCH

The effects of boric acid on sister chromatid exchanges and chromosome aberrations in cultured human lymphocytes

Mehmet Arslan · Mehmet Topaktas · Eyyüp Rencuzogullari

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Abstract The aim of this study was to determine the possible genotoxic effects of boric acid (BA) (E284), which is used as an antimicrobial agent in food, by using sister chromatid exchange (SCEs) and chromosome aberration (CAs) tests in human peripheral lymphocytes. The human lymphocytes were treated with 400, 600, 800, and 1000 μg/mL concentrations of BA dissolved in dimethyl sulfoxide (DMSO), for 24 h and 48 h treatment periods. BA did not increase the SCEs for all the concentrations and treatment periods when compared to control and solvent control (DMSO). BA induced structural and total CAs at all the tested concentrations for 24 and 48 h treatment periods. The induction of the total CAs was dose dependent for the 24 h treatment period. However, BA did not cause numerical CAs. BA showed a cytotoxic effect by decreasing the replication index (RI) and mitotic index (MI). BA decreased the MI in a dose-dependent manner for the 24 h treatment period.

Keywords Human peripheral lymphocytes · Boric acid · SCE · CA

M. Arslan
Department Biology, Natural and Applied Science Institute, Çukurova University, 01330 Adana, Turkey

M. Topaktas · E. Rencuzogullari (✉)
Department Biology, Faculty of Science and Letters, Çukurova University, 01330 Adana, Turkey
e-mail: reyyup@cu.edu.tr

Introduction

The population of the world is increasing every year. According to a United Nations report, the population of the world reached 6 billion in 1999, and an estimated 9 billion people will live on the planet by 2050. Therefore, it is essential to find new food sources and preserve them for a long period. Because of this, many of the methods used for storing food for a long period have been improved, and many chemical substances have been used as antimicrobial agents for the preservation of food. It has been reported that most of these chemical substances, especially the antimicrobial agents, are genotoxic in various test systems (Mukherjee et al. 1988; Meng and Zhang 1992; Rencuzogullari et al. 2001a; Rencuzogullari et al. 2001b; Blaszczyk et al. 2003; Poul et al. 2004). However, there are many food preservatives for which the genotoxic effects are still unknown.

Boric acid (BA) (E284) is used as a food preservative in products such as caviar (Ministry of Agricultural of Turkey 2004) and is used for both medicinal and nonmedicinal purposes (Heindel et al. 1997). Ommaty (2000) reported that BA is also used as an antiseptic agent against skin infections. The Ministry of Agriculture (of Turkey) (2004) suggested that BA may be used at a maximum dose of 4 g/L (4000 mg/L) in food as an antimicrobial substance. BA adversely affected reproduction and fertility in rodents (Heindel et al. 1997), and showed

a higher degree of developed mutagenicity in the presence of gamma rays and fast neutrons in Chinese hamster ovary (CHO) cells (Kinashi et al. 1997). However, BA was not identified as a mutagen in mouse lymphoma cells (McGregor et al. 1988). BA was not mutagenic in the Salmonella/microsome test system and also did not increase the sister chromatid exchanges (SCEs) in Chinese hamster ovary (CHO) cells (National Toxicology Program 1987). According to a European Food Safety Authority (EFSA) report (2004), BA caused adverse effects in animals after long-term toxicity studies. In addition, the EFSA (2004) noted that boric acid impaired fertility, although BA was not mutagenic in prokaryotic and eukaryotic test systems according to limited genotoxicity studies.

There are several long- and short-term studies on the adverse, developmental, and reproductive effects of BA. However, there are limited studies on the carcinogenicity and genotoxicity of boric acid. On the other hand, there are no published data on the effects of BA on the SCEs and chromosome aberrations (CAs) in human peripheral lymphocytes. BA was negative in in vitro assay for CA and SCE in CHO cells (National Toxicology Program 1987). However, Madle et al. (1986) reported that Chinese hamster was not usable for detecting the genotoxicity of aflotoxin and that the choice of an inappropriate test species may lead to a false-negative result on the genotoxic potential of compounds. In addition, Madle et al. (1993) reported that the use of human lymphocytes for the mutagenicity studies could produce the best results for humans. To consider the genotoxicity of BA, its genotoxicity towards human lymphocytes should be investigated. For this reason, the aim of this study was to investigate the genotoxic effects of BA in human lymphocytes.

Materials and methods

The techniques of Evans (1984) and Perry and Thompson (1984) were followed for the preparation of chromosomes, with minor modifications. The study was designed to follow IPCS guidelines (Albertini et al. 2000).

Whole blood (0.2 mL) from four healthy donors (two male and two female, nonsmokers, aged 27–30 years, blood samples not pooled) was added to 2.5 mL chromosome medium B (Biochrom, F5023) supplemented with 10 μg/mL bromodeoxyuridine (Sigma, B5002). The medium contains phytohaemagglutinin (PHA) for stimulating the proliferation of the cells. The cultures were incubated at 37°C for 72 h, and then treated with 400, 600, 800, and 1000 μg/mL concentrations of BA dissolved in dimethyl sulfoxide (DMSO), for 24 h (BA added 48 h after initiating culture) and 48 h (BA added 24 h after initiating culture). A negative control, a solvent control (DMSO, 10 μL/mL), and a positive control (mitomycin-C, MMC, 0.25 μg/mL, Kyowa Hakko, Japan) were also used. DMSO is a good solvent that did not induce CA, and induced a small amount of SCE. Colchicine (0.06 μg/mL, Sigma C9754) was present for the last 2 h of culture. To collect the cells, the cultures were centrifuged (1200 rpm, 15 min), treated with hypotonic solution (0.4% KCl) for 13 min at 37°C, and then fixed in cold methanol:glacial acetic acid (3:1) for 20 min at room temperature. Treatment with fixative was repeated three times, then the cells were spread on glass slides and air-dried. The slides were stained with Giemsa according to the fluorescence plus Giemsa technique (Speit and Haupter 1985).

The number of CA was obtained by calculating the percentage of metaphases from each concentration and treatment period that showed the structural and numerical alterations. The CA was classified according to the international system for human cytogenetic nomenclature (ISCN) (Paz-y-Mino et al. 2002). A hundred well-spread metaphases per donor (a total of 400 metaphases per concentration) were examined at 1000× magnification for the occurrence of the CA. Gaps were not counted as CA, according to Mace et al. (1978). SCE scoring was carried out according to Albertini et al. (2000). For the number of SCEs, a total of 100 cells (25 cells from each donor) under second metaphases were examined. The results were used to determine the mean number of SCEs (SCE/cell). In addition, a total of 400 cells (100 cells from each donor) were scored for the determination of the replication index (RI). The mitotic index (MI) was also determined by scoring 3000 cells from each donor. The MI explains the effects of the chemicals on the G2 stage of the cell cycle, and the RI reflects the effects of the chemicals on the S and G2 stages of the cycle. The RI was calculated according to the

following formula: RI = (M1 × 1) + (M2 × 2) + (M3 × 3)/total scored cells, where M1, M2, and M3 are the fraction of cells undergoing their first, second, and third mitosis during the 72-h cell culture period.

The significance of differences between the mean SCEs, RI, MI, structural, numerical, and total CAs in the treated cultures and their controls were determined using the *t*-test. Dose-response relationships were determined from the correlation and regression coefficients for the mean SCEs, RI and MI, structural, numerical, and total CAs.

Results

The human lymphocytes were treated with 400, 600, 800, and 1000 μg/mL concentrations of BA dissolved in DMSO, for 24 h and 48 h treatment periods to investigate the effects of BA on SCEs and CAs in human lymphocytes. Table 1 shows the effect of BA on SCEs, RI, and MI. BA did not statistically significantly increase the frequency of SCEs when compared to the control and solvent control. SCEs were not evaluated at the highest concentrations of BA (1000 μg/mL) for the 48 h treatment period due to the excessive toxicity of BA. However, BA decreased the RI at all concentrations for both treatment periods, except the 600 μg/mL concentration for 24 h. In addition, BA decreased the MI in a dose dependent way for the 24 h treatment period ($r = -0.94$) (Table 1).

BA induced structural chromosome aberrations at all concentrations after the 24 h and 48 h treatment periods. However, BA did not induce numerical chromosome aberrations. In addition, BA induced total chromosome aberrations at all concentrations and treatment periods. BA also induced total CAs in a dose-dependent manner for the 24 h treatment period ($r = 0.98$). As shown in Table 2, BA was able to induce structural CAs instead of numerical CAs. Chromatid and chromosome type were the most common abnormalities.

The test substance BA did not changed the pH of the medium at the highest concentration (1000 μg/mL). The pH of the untreated culture was measured as 7.29 while the pH of the treated culture with a 1000 μg/mL concentration of BA was measured as 7.20.

Discussion

In the present study, BA did not increase the frequency of SCEs. However, BA induced CAs in a

Table 1 Sister chromatid exchanges, replication index, and mitotic index in cultured human lymphocytes treated with BA

Test substance	Treatment		Min.-max. SCE	SCE/cell ± SE	RI ± SE	MI ± SE
	Periods (h)	Concentrations (μg/mL)				
Control	–	–	1–17	7.46 ± 0.89	2.42 ± 0.07	3.24 ± 0.37
DMSO	24	10 μL/mL	1–20	8.23 ± 0.96	2.38 ± 0.18	3.02 ± 0.38
MMC	24	0.25	8–63	30.7 ± 8.040	1.44 ± 0.06	2.19 ± 0.59
BA	24	400	2–19	7.36 ± 0.75	2.25 ± 0.05 a_1	3.00 ± 0.25
		600	2–18	7.68 ± 0.57	2.20 ± 0.11	2.29 ± 0.18 a_1b_1
		800	2–17	7.74 ± 0.90	2.21 ± 0.07 a_1b_1	2.14 ± 0.24 a_1b_1
		1000	2–19	7.43 ± 1.23	2.09 ± 0.12 a_1	1.89 ± 0.65 a_1
DMSO	48	10 μL/mL	2–23	8.74 ± 0.91	2.25 ± 0.14	2.46 ± 0.52
MMC	48	0.25	24–104	66.3 ± 11.09	1.38 ± 0.07	2.79 ± 0.44
BA	48	400	0–16	7.88 ± 1.24	1.46 ± 0.08 a_3b_3	1.70 ± 0.25 a_2b_1
		600	2–9	7.88 ± 1.28	1.22 ± 0.05 a_3b_3	1.73 ± 0.28 a_1b_1
		800	5–22	9.50 ± 2.30	1.06 ± 0.008 a_3b_3	1.13 ± 0.12 a_3b_2
		1000*	–	–	1.12 ± 0.05 a_3b_3	0.64 ± 0.14 a_3b_2

*: Not scored due to insufficient cells for SCE due to excessive toxicity

a, significant difference from control; b, significant difference from solvent (DMSO) control

a_1b_1: $p < 0.05$; a_2b_2: $p < 0.01$; a_3b_3: $p < 0.001$

Table 2 Structural, numerical, and total chromosome aberrations in cultured human lymphocytes treated with BA[+]

Test substance	Treatment		Structural CA			Numerical CA ± SE (%) (P+E)*	Total CA ± SE (%)
	Periods (h)	Concentration (μg/mL)	Chromatid	Chromosome type	Total ± SE (%)		
Control	–	–	9	7	4.00 ± 0.91	–	4.00 ± 0.91
DMSO	24	10 μL/mL	11	8	4.75 ± 0.62	–	4.75 ± 0.62
MMC	24	0.25	50	56	31.00 ± 0.40	–	31.00 ± 0.40
BA	24	400	19	10	7.25 ± 0.94 a_1	0.25 ± 0.25	7.50 ± 0.86 a_1b_1
		600	22	13	8.75 ± 0.75 a_2b_1	–	8.75 ± 0.75 a_2b_1
		800	27	17	11.00 ± 1.68 a_1b_1	–	11.00 ± 1.68 a_1b_1
		1000	22	24	11.50 ± 0.86 a_2b_2	0.50 ± 0.28	12.00 ± 0.57 a_3b_3
DMSO	48	10 μL/mL	5	22	6.75 ± 0.47	–	6.75 ± 0.47
MMC	48	0.25	150	116	66.25 ± 1.84	–	66.25 ± 1.84
BA	48	400	29	22	13.50 ± 1.75 a_1b_1	0.50 ± 0.28	14.00 ± 1.47 a_2b_1
		600**	22	11	11.33 ± 1.85a_1	–	11.33 ± 1.85 a_1
		800**	37	17	23.00 ± 3.67 a_1b_1	–	23.00 ± 3.67 a_1b_1
		1000***	20	13	29.00 ± 4.50 a_1b_1	–	29.00 ± 4.50 a_1b_1

[+] A total of 400 cells were scored

* P, polyploid; E, endoreduplication

** Only 270 cells and *** 150 cells were scored due to excessive toxicity

a, significant difference from control; b, significant difference from solvent (DMSO) control

a_1b_1: $p < 0.05$; a_2b_2: $p < 0.01$; a_3b_3: $p < 0.001$

dose dependent way. According to the report of the National Toxicology Program (1987), BA was not mutagenic in the Salmonella/microsome test system and also BA did not increase the SCEs in Chinese hamster ovary (CHO) cells. These results support the results obtained from this study on SCEs. However, in this study BA induced CAs. BA can lead to the formation of CAs by breaking the phosphodiester backbone of DNA. CA formation occurred at all the tested BA concentrations. Dose-dependent induction of CAs showed that BA most probably poses a genotoxic risk to human peripheral lymphocytes. Heindel et al. (1997) reported that BA was a potent reproductive toxicant in male and female mice. BA was not identified as a mutagen in lymphoma cells (McGregor et al. 1988). However, BA showed a synergistic effect on the induction of mutagenicity when used together with both gamma rays and fast neutrons in CHO (Kinashi et al. 1997) and enhanced DNA damage in human melanoma cells using the comet assay (Poller et al. 1996). Hubbard (1998) reported that inorganic borates including boric acid, Na, ammonium, K, and Zn borates, generally display low acute toxicity orally, dermally, and by inhalation. Hubbard (1998) also reported that no effect on fertility was seen in a population of workers exposed to borates, and in a population exposed to high environmental borate levels. BA induced beta-galactosidase synthesis in the *E. coli* PQ37 strain both in the presence and absence of the S9 mix (Odunola 1997). Therefore, BA may not require metabolic activation to be genotoxic in bacteria (Odunola 1997).

BA had a cytotoxic effect, decreasing both the RI and the MI, in human lymphocytes. Donbak et al. (2002) and Turkoglu (2006) reported that BA decreased the MI and caused mitotic abnormalities in *Allium cepa* root tip cells. This result also supports the cytotoxicity of BA. The decreasing of the MI or the inhibition of DNA synthesis might be caused by decreasing adenosine triphosphate (ATP) level and pressure from the functioning of the energy-production centre (Epel 1963; Jain and Andsorbhoy 1988).

There are some contrary results about the genotoxicity of BA, which may have arisen from the doses, treatment method, and test material used. The animals that were used for the tests may also lead to the different results, as described early by Madle et al. (1986).

BA can be described as an in vitro mutagen according to the somatic cells test. Lang and Madle (1993) reported that the mutagenicity of substances could be explained without using gametic test protocols because substances that are mutagens for somatic cells could also be determined as mutagens for gametic cells. Basler (1993) also reported that oocytes were not suitable to be used in a routine test protocol for genotoxicity studies because of the limited ovulation of the oocytes, with only 10 oocytes being ovulated from each animal (mice or hamster) (Basler 1993). It is impossible to score chromosome abnormalities and carry out statistical analysis with this limited number of oocytes (Basler 1993). According to these results, it could be concluded that somatic cell tests might give better results than gametic cell tests. On the other hand, Treinen and Chapin (1991) reported that boron levels had effectively reached the testis, inhibited spermiation, caused epithelial disorganization, and germ cell loss in adult F344 male rats. Therefore, Ku and Chapin (1994) reviewed the mechanism of testicular toxicity of BA and reported that BA caused testicular lesions in adult rats characterized by inhibited spermiation that may progress to atrophy. BA caused atrophy by affecting the Sertoli cell energy metabolism and impaired the nucleic acid synthesis in liver and in testis (Ku and Chapin 1994). As seen, BA had a teratogenic effect, could reach the testis, and caused atrophy and germ cells loss. Parry (2000) reported that, for most chemicals recognized as in vivo somatic cell mutagens, no further genotoxicity testing is necessary since they will be assumed to be potential genotoxic carcinogens and potential germ cell mutagens. Only in some specific cases may germ cell studies be undertaken to demonstrate that a somatic cell mutagen is not a gametic cell mutagen. Teratogenic studies could be constructed instead of germ cell studies (Parry 2000). Walmod et al. (2004) reported that BA inhibited proliferation in a linear dose-dependent manner, caused cytotoxicity, and also had a teratogenic effect in murine fibroblast L929 cells. According to these results it can be concluded that BA is capable of causing mutation in gametic cells.

The test substance BA did not changed the pH of the medium at the highest concentration (1000 μg/mL). It was reported that a deviation from neutral pH can lead to genotoxic effects (Siddique et al. 2005; Jenkins et al. 2007).

As a result, BA displayed toxicity and a synergistic effect on the induction of mutagenicity when used together with fast neutrons and gamma rays. However, it was not mutagenic for Salmonella test strains. BA induced CAs and decreased the RI and the MI in human lymphocytes without changing the pH of the medium. BA was also capable of reaching the testis and was capable of causing mutation in gametic cells. Therefore, it can be concluded that BA might pose a potential risk for humans. However, it must be investigated in other test systems for its in vivo genotoxic effects.

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