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Boric acid as a protector against paclitaxel genotoxicity

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Paclitaxel (PAC) is an anticancer drug used for treatments of breast, ovarian and lung cancers. However, little data is available in the literature on its potential genotoxicity on healthy human cells. On the other hand, boron deficiency and supplementation exert important biological effects in human and animal tissues. The biological effects of dietary boron are defined, but its interaction with PAC is not known for therapeutic uses. The aim of the present study was to determine whether boric acid (BA) confer a protection against PAC genotoxicity. After the application of PAC (10 or 20 μg/l) and BA (2.5 or 5 mg/l), the genotoxic effects were assessed by sister chromatid exchange (SCE) and micronucleus (MN) tests in human blood cultures. We also analyzed nuclear division index (NDI) in peripheral lymphocytes. Our results showed that PAC significantly ($P < 0.05$) increased the frequencies of SCEs and the formations of MNs in peripheral lymphocytes as compared to controls. PAC decreased the nuclear division index in lymphocyte cultures. Boric acid did not show cytotoxic or genotoxic effects at the concentrations tested. Furthermore, the PAC-induced increases in the genotoxicity and cytotoxicity indices were diminished by the addition of BA. The present study suggests for the first time that BA can prevent the genotoxicity of PAC on human lymphocytes.

Keywords: boric acid, paclitaxel, sister chromatid exchange, micronucleus, nuclear division index

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INTRODUCTION

Paclitaxel (PAC), an anticancer drug, is used for the treatments of breast, ovarian, lung, head and neck cancers (Von Hoff, 1997; Rowinsky, 1997). However, this drug is known to cause adverse effects in different organs such as heart and brain (Lachkar *et al.,* 2006). Therefore, numerous investigations have been performed to find protective agents minimizing the adverse effects of PAC (Della Torre, 1999; Kanat *et al.,* 2003; Openshaw *et al.,* 2004; Chentanez *et al.,* 2009). The generation of reactive oxygen species (ROS) increases in cells after PAC treatment. The increase in ROS production can be suppressed by antioxidants (Wang *et al.,* 2004). Some studies showed that boron compounds have nutritional benefits such as increased vitamin D biosynthesis and induction of hematopoiesis, and the ability to strengthen the tissue antioxidant defenses in animals and humans (Hunt & Idso, 1999; Pawa & Ali, 2006). In addition, it has been reported that boric acid (BA) is a non-genotoxic agent increasing the antioxidant capacity of cultured human blood cells (Turkez *et al.,* 2007; Turkez, 2008). To our best knowledge, there has been no report about the role of BA on PAC-treated cultures. In this study, we investigated the protective effect of BA in human lymphocyte cell cultures treated with PAC. The sister chromatid exchange (SCE) and micronucleus (MN) tests, considered to be sensitive methods for detecting DNA damage (Wu & Wu, 1995), were used to assess the genetic damage.

MATERIAL AND METHODS

Cell cultures. Whole blood samples were collected from two healthy non-smoker donors with no history of exposure to any genotoxic agent. Cultures were set up according to the protocol described by Evans and O'Riordan (1975) with a slight modification. The peripheral blood lymphocytes (0.5 ml) were cultured in 5 ml of culture medium (Chromosome Medium B, Biochrom®) with phytohemagglutinin. PAC (10 or 20 μg/l) (taxol, Bristol-Myers Squibb) and BA (2.5 or 5 mg/l) (Sigma) were added alone or together to the cultures except control group just before incubation.

SCE (sister chromatid exchange) test. To determine the frequency of SCE in the lymphocytes, BrdU (5-bromo-2'-deoxyuridine, Sigma, MO, USA) was added to each culture (10^{-4} M final concentration) and the cultures were incubated in complete darkness for 72 h at 37 °C. Colchicine (0.5 μg/ml, Sigma) was added to each culture during the last 2 h of the incubation to block the cells in metaphase. After the incubation, the cultures were harvested by a procedure including hypotonic treatment (0.075 M KCl) for 25 min followed by three repetitive cycles of fixation in methanol/acetic acid solution (3:1, v/v). The cell suspension was dropped onto cold microscopic slides. The slides were dried at room temperature and complete darkness for three days. They were differentially stained for inspection of the SCE rate according to the fluorescence plus Giemsa (FPG) procedure (Rooney & Czepulkowski, 1986). For each treatment condition, 20 well-spread second division metaphases containing 46 chromosomes in each cell were scored by using an Olympus BH-2 microscope (Olympus Corp., Tokyo, Japan) and the values obtained were calculated as SCEs per cell.

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Abbreviations: BA, boric acid; BrdU, 5-bromo-2′-deoxyuridine; FPG, fluorescence plus Giemsa; MN, micronucleus; NDI, nuclear division index; PAC, paclitaxel; SCE, sister chromatid exchange.

Micronucleus (MN) test and Nuclear Division Index (NDI) analysis. In order to detect the number of micronucleated lymphocytes, cytochalasin B (4.5 µg/ml, Sigma) were added to the cultures at 44th hour. At the end of the 72 h incubation period, the lymphocytes was treated with 0.075 M KCl for 8 min at 37°C. After three fixation cycles with methanol/acetic acid (396:1, v/v), cell suspension was dropped onto cold slides. The slides were air-dried at room temp. and then stained with 5% Giemsa for 15 min. All slides were coded before scoring. The criteria for scoring micronuclei were as described by Fenech (1993). At least 2000 binucleated lymphocytes per concentration were examined for the presence of one, two or more micronuclei.

For cell cycle analysis, 400 cells per treatment group were scored for the presence of one, two or more than two nuclei and the nuclear division index (NDI) was calculated as follows:

$$NDI = [1N + (2 \times 2N) + (4 \times >2N)]/C$$

where 1N is number of cells with one nucleus, 2N — with two nuclei, and >2N — with more than two nuclei, C — number of cells examined (Konopacka & Rogolinski, 2004).

Statistical analysis. The statistical analysis of experimental values in the SCE and MN tests was performed by Student's *t*-test and using the S.P.S.S. 12.0 software. Statistical decisions were made with a significance level of 0.05.

RESULTS

Our results showed that BA (at either dose) did not alter SCE and MN frequencies in human lymphocytes. PAC at 10 and 20 µg/l significantly increased the SCE rates and MN formation in lymphocytes as compared with controls (Figs. 1 and 2). When the two agents were used, BA reduced the number of PAC-induced SCE and MN formation but it did not completely revert the PAC-caused SCE and MN induction.

PAC at 20 µg/l also reduced the NDI in human lymphocytes *in vitro* (Fig. 3). Addition of BA to the cultures brought back the NDI rates to the control value.

DISCUSSION

Our results clearly indicated the PAC induced genotoxic and cytotoxic damage in human lymphocytes. Similarly to our findings, a few reports indicated *in vitro* paclitaxel genotoxicity. Preisler *et al.* (1999) investigated the genotoxic effects of a combination of PAC and radiation in four mammalian cell lines including L5178Y, V79, HeLa and breast cancer cell line MCF-7 by the MN assay. They showed that the MN frequencies in combined treatments were different from a presumed additive effect of the single treatments. PAC was found to be a strong *in vitro* aneugenic drug in human normal cells at therapeutic doses (Digue *et al.,* 1999). In another report, PAC significantly increased the micronucleated rates in a concentration-dependant manner in healthy human lymphocytes (Digue *et al.,* 2002). In contrast to those *in vitro* results, Cunha *et al.* (2001) reported that paclitaxel was non-genotoxic in *Drosophila* wing somatic mutation and recombination test (SMART). However, PAC appeared as cytotoxic clastogene in mouse lymphoma cells by using comet and apoptotic assays (Lee *et al.,* 2003). And

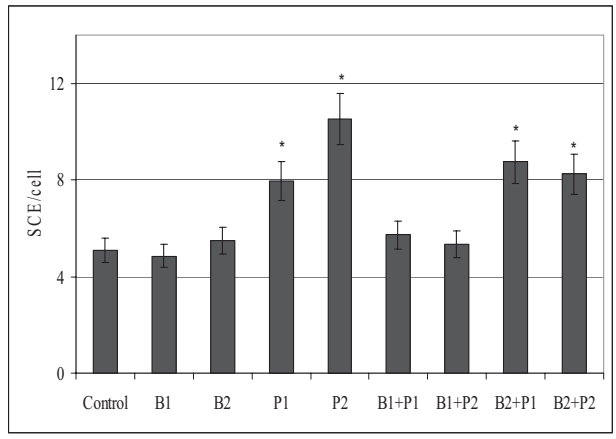

Figure 1. SCE frequencies in cultured human lymphocytes treated with PAC and BA
B1=2.5 mg/l BA; B2=5 mg/l BA; P1=10 µg/l PAC; P2=20 µg/l PAC; *represents statistically significant differences from control group ($P<0.05$). Values are means ± standard deviation.

the cytotoxic activity of PAC was suggested partially due to its ability to induce apoptosis *via* activation of cell cycle dependent kinases leading to activation of caspases (Moos & Fitzpatrick, 1998) and then further to DNA fragmentation (Ellinger-Ziegelbauer *et al.,* 2009). This is in line with previously published data and PAC was found to be genotoxic in normal non-human primate bone marrow cells (Rao *et al.,* 2005). We also found a significant decrease in the rate of NDI after the treatment with high PAC dose, which could be due to its anti-proliferative action *in vitro* (Axel *et al.,* 1997). As a matter of fact, PAC has been shown to inhibit cell cycle progression and to induce cell cycle arrest prior to the induction of apoptosis in cancer cells by binding to microtubules (Aoudjit & Vuori, 2001).

The present study also demonstrated that the reduction of PAC induced SCE and MN formation was caused by the protective effect of BA. Earlier studies showed that BA did not lead to DNA damage in lymphocytes (Turkez *et al.,* 2007). Moreover, this compound significantly decreased genotoxic effects of some agents such as titanium and vanadium (Turkez, 2008; Geyikoglu & Turkez, 2008). This protective mechanism of BA is unclear but it can be due to its ability to strengthen the

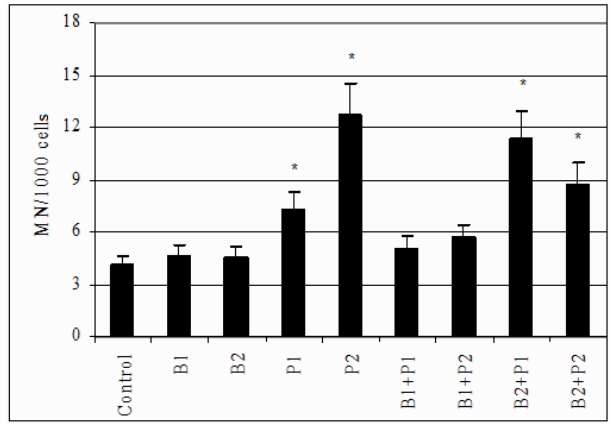

Figure 2. Rates of MN in cultured human lymphocytes treated with PAC and BA
Abbreviations are as in Fig. 1.

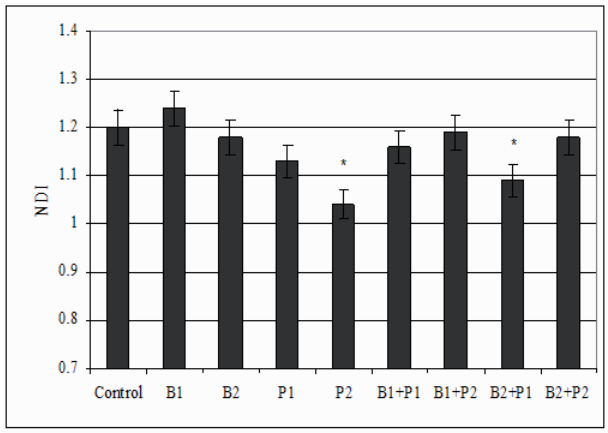

Figure 3. Rates of NDI in cultured human lymphocytes treated with PAC and BA
Abbreviations are as in Fig. 1.

tissue antioxidant capacity and defense against free oxygen radicals. In line with that, a study by Ramanathan *et al.* (2005) indicated that reactive oxygen and nitrogen species were involved in PAC cytotoxicity. Thus, BA could modulate the PAC-induced genetic and cytotoxic damage by preventing free radical generation or by stimulating components of the antioxidant defense system. In addition, our results revealed that 2.5 mg/l of BA had a stronger protective effect than 5 mg/l. The reason could be that the higher dose (5 mg/l) used was beyond the physiological limits. In fact, normal value of BA in the blood has been reported as 3 mg/l (EVM, 2002, Expert Group on Vitamins and Minerals. Revised review of boron. EVM/99/23/P.REVISEDAUG2002). BA also loses its protective action and becomes toxic at doses above physiological limits (Turkez *et al.,* 2007).

In conclusion, our results clearly indicated that boron (as BA) supplementation to lymphocyte cultures ameliorated the PAC- induced DNA damage. BA could play a similar role in cancer patients after chemotherapy. However, specific protective agents for chemotherapy-induced adverse effects after PAC or other anti-cancer drugs treatment should not show any interference with the antitumor activity of the drugs (Pisano *et al.,* 2003), and the *in vivo* interactions of BA and PAC are still unknown. At this point, further *in vivo* investigations are necessary to justify daily intake of boron to minimize adverse effects of anti-cancer drugs.

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