Environmental Toxicology and Pharmacology 26 (2008) 342–347

Contents lists available at ScienceDirect

Environmental Toxicology and Pharmacology

journal homepage: www.elsevier.com/locate/etap

# Boron compounds reduce vanadium tetraoxide genotoxicity in human lymphocytes

Fatime Geyikoglu*, Hasan Turkez

*Department of Biology, Faculty of Arts and Sciences, Atatürk University, Erzurum 25240, Turkey*

ARTICLE INFO

*Article history:*
Received 17 January 2008
Received in revised form 1 June 2008
Accepted 7 July 2008
Available online 12 July 2008

*Keywords:*
Borax
Boric acid
Genotoxicity
Human lymphocytes
Total antioxidant capacity
Vanadium

ABSTRACT

Vanadium has potential medical and pharmacological uses although it may also show genotoxic effects. Biological effects of boron are defined, but its interaction with vanadium is not known for therapeutic uses. The objective of present study was especially to determine whether boron compounds (boric acid and borax) conferred the protection against vanadium(IV) tetraoxide genotoxicity. After the application of vanadium (5, 10 and 20 mg/l) and boron compounds (5 and 10 mg/l), blood cultures were assessed by genetic endpoints and total antioxidant capacity (TAC). According to our results, vanadium(IV) tetraoxide induced a reduction in proliferation index (PI). Besides, the frequencies of sister-chromatid exchanges (SCEs), micronuclei (MN) rates and chromosomal aberrations (CAs) in peripheral lymphocytes were significantly increased by vanadium(IV) tetraoxide (10 and 20 mg/l) compared to controls. On the other hand, boric acid and borax did not show cytotoxic and genotoxic effects at the concentrations tested. Moreover, these compounds elevated TAC in erythrocytes. The order of anti-genotoxicity efficacy against vanadium was boric acid and borax, respectively. In conclusion, boron compounds have been shown to protect vanadium-induced DNA damage *in vitro* for the first time.



## 1. Introduction

Vanadium is a transition metal that is emitted to the atmosphere during the combustion of fossil fuels (Aragon et al., 2005). The main source of vanadium intake for the general population is food (Rodriguez-Mercado et al., 2003). Interest in vanadium compounds is because of their toxic effects and uses in some biomedical areas such as an antineoplastic, cholesterol and glucose level blood, diuretic, oxygen hemoglobin affinity (Altamirano-Lozano et al., 1998; Sakurai, 2005). Vanadium tetraoxide is an inorganic chemical species in (IV) oxidized form that has been shown to induce toxic effects *in vitro* and *in vivo* models (Aragon and Altamirano-Lozano, 2001; Rodriguez-Mercado et al., 2003). Vanadium caused the liver injury in cultured hepatocytes (Barreto et al., 2005). Vanadium has also the potential to exert tumorigenic/carcinogenic activity (Valko et al., 2005). It induces gene conversion in yeast. However, the data on the genotoxic potential of vanadium compounds in bacterial systems are inconclusive (Altamirano-Lozano et al., 1998). Likewise, the effects on the SCE and CA frequencies of these compounds in human lymphocytes are limited and controversial (Rojas et al., 1996).

Boron is a trace mineral for plants, animals and humans. It also plays an important role in improving arthritis, plasma lipid profiles and brain function (Devirian and Volpe, 2003). Borates (general term associated with boron containing minerals) most commonly originate in dried salt lakebeds of desert or arid areas. Of all the borates, borax and boric acid are the only two minerals which are utilized for commercial applications. These compounds show minimal potential for genotoxicity in bacteria and cultured mammalian cells (Moore and Expert Scientific Committee, 1997).

The tissues retain vanadium mainly as vanadium(IV) (Aragon et al., 2005). And the blood cells are used to describe the genotoxic effects of vanadium tetravalent compounds (Migliore et al., 1993, 1995; Rodriguez-Mercado et al., 2003). To be clear in basic toxicity information, it is necessary to carry out more studies in the field of genetic toxicology with vanadium. Thus, considerable attention has attracted in recent years the assessment of the genotoxicity of vanadium compounds (Villani et al., 2007). With the present study was evaluated genotoxic (SCE, CA and MN assays), cytotoxic (PI) and biochemical effects (TAC) of vanadium(IV) tetraoxide in human blood. Besides, efforts have been made to minimize the toxic effect caused by vanadium compounds (Barreto et al., 2005). Therefore, this study especially investigated the ability of boron compounds (boric acid and borax) to reduce genotoxicity of vanadium.

* Corresponding author at: Ataturk Universitesi, Fen-Edebiyat Fakultesi, Biyoloji Bolumu, 25240 Erzurum, Turkey. Tel.: +90 442 231 43 25; fax: +90 442 236 09 48.
*E-mail address:* geyikogluff@yahoo.com (F. Geyikoglu).

1382-6689/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.etap.2008.07.002

## 2. Materials and methods

Human blood was obtained by veinpuncture from four non-smoking donors. Vanadium(IV) tetraoxide was purchased from Sigma Chemical Co (USA) and two boron compounds (boric acid and borax) were purchased from Eti Mine Works General Management (TURKEY). Vanadium(IV) tetraoxide (in concentrations of 5, 10 and 20 mg/l) and boron compounds (in concentrations of 5 and 10 mg/l) were dissolved in water. The compounds were added to the cultures just before incubation for cytogenetic and biochemical analysis as mentioned below. Experiments conformed to the guidelines of the World Medical Assembly (Declaration of Helsinki). These investigations stem from the works of Rodriguez-Mercado et al. (2003), Türkez and Geyikoglu (2006) and Türkez et al. (2007).

### 2.1. Cytogenetic analysis

#### 2.1.1. SCE assay and proliferation index

Cultures were set up according to a slight modification of the protocol described by Evans and O'Riordan (1975). A 0.5 ml aliquot of heparinized blood was cultured in 6 ml of culture medium (Chromosome Medium B, Biochrom, Berlin) with 5 μg/ml of phytohemagglutinin (Biochrom). Above-mentioned doses of vanadium and boron compounds were added separately to the culture tubes just before incubation. In addition, these compounds were also added together to the cultures to test an antimutagenic activity. On the other hand, the negative control samples were incubated without vanadium and boron compounds. And, Mitomycin C (Sigma, 0.6 μg/ml) was used as a positive control. With the aim of providing successive visualization of SCEs, 5-bromo-2′-deoxyuridine (BrdU) (Sigma, 20 μM) was added after culture initiation. The blood cultures were incubated in complete darkness for 72 h at 37 °C. Exactly 70 h and 30 min after beginning incubations, demecolcine (*N*-diacetyl-*N*-methylcolchicine, Sigma) was added to the medium to achieve a final concentration of 0.5 μg/l. After hypotonic treatment (0.075 M KCl), followed by three repetitive cycles of fixation in methanol/acetic acid solution (3:1, v/v), centrifugation, and resuspension, the cell suspension was dropped onto chilled, grease-free microscopic slides, air-dried, aged, and then stained for the inspection of SCE rate with fluorescence plus Giemsa (FPG). For each treatment condition, well-spread thirty second-division metaphases containing 42–46 chromosomes in each cell were scored, and the values obtained were calculated as SCEs per single cell.

In addition to SCEs, cells were analyzed for the relative frequency of first-division metaphases (M1; identifiable by uniform staining of both sister-chromatids), second-division metaphases (M2; identifiable by differential staining of the sister-chromatids), and third- and subsequent division metaphases (M3; identifiable by nonuniform pattern of staining). PI is the average number of replications completed by metaphase cells and is calculated as: $PI = 1 \times (\%M1) + 2 \times (\%M2) + 3 \times (\%M3)/100$.

#### 2.1.2. Chromosomal aberrations assay

For analysis of structural CAs (chromatid or chromosome gaps and chromatid or chromosome breaks) parallel cultures were carried out without BrdU for 72 h. Treatments were similar as described in SCEs analysis. 2 h prior to harvesting 0.1 ml of demecolcine was added to the culture tube. Hypotonic treatment and fixation were performed in the same way as in SCE analysis. To prepare slides, 3–5 drops of the fixed cell suspension were dropped on a clean slide and air-dried. The slides were stained in 3% Giemsa solution in phosphate buffer (pH 6.8) for 15 min. Thirty well-spread metaphases were analyzed for each treatment to detect the presence of chromosomal aberrations as CAs per single cell. Criteria to classify the different types of aberrations were in accordance with the recommendation of EHC 46 for environmental monitoring of human populations (IPCS, 1985).

#### 2.1.3. Micronucleus assay

The MN test was performed by adding cytochalasin B (Sigma; final concentration of 6 μg/ml) after 44 h of culture. At the end of the 72-h incubation period, the lymphocytes were fixed with ice-cold methanol:acetic acid (1:1). The fixed cells

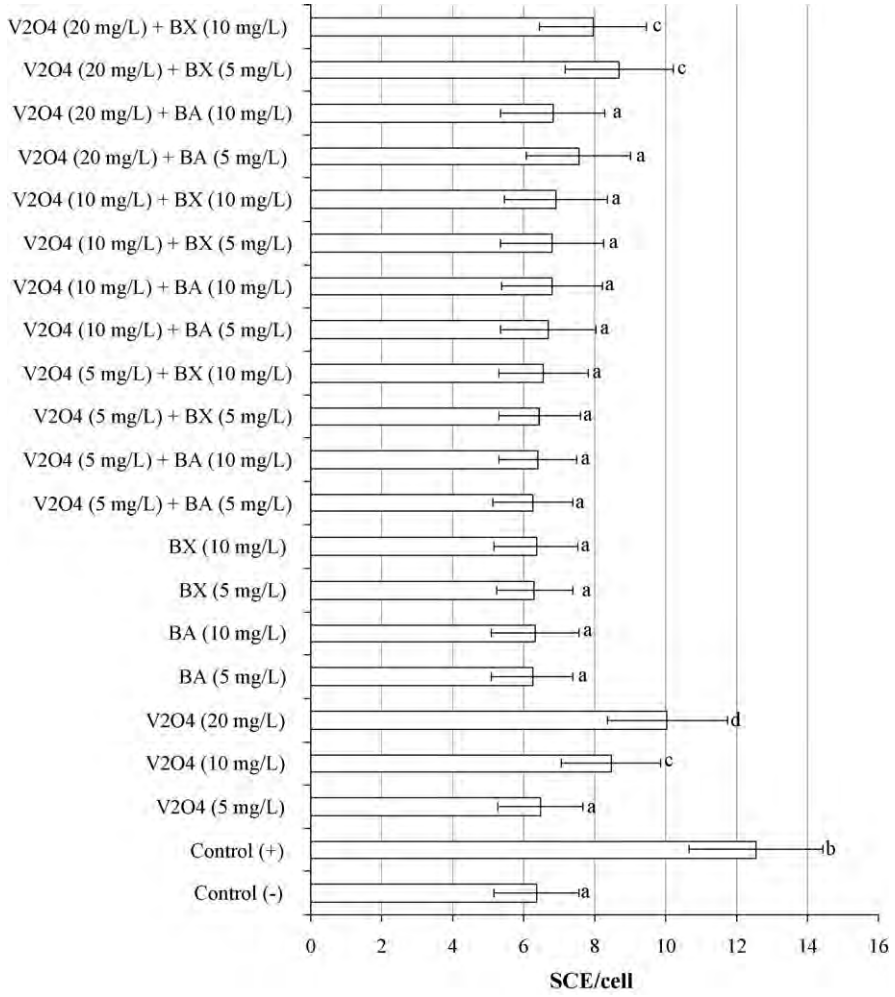


**Fig. 1.** The frequencies of SCEs in human blood cultures treated with different concentrations of vanadium(IV) tetraoxide and the two boron compounds. Control (−) = negative control; control (+) = positive control (Mitomycin C); BA = boric acid; BX = borax; different letters above each bar represent statistically significant differences between groups ($P = 0.05$). Values are means ± standard deviation.

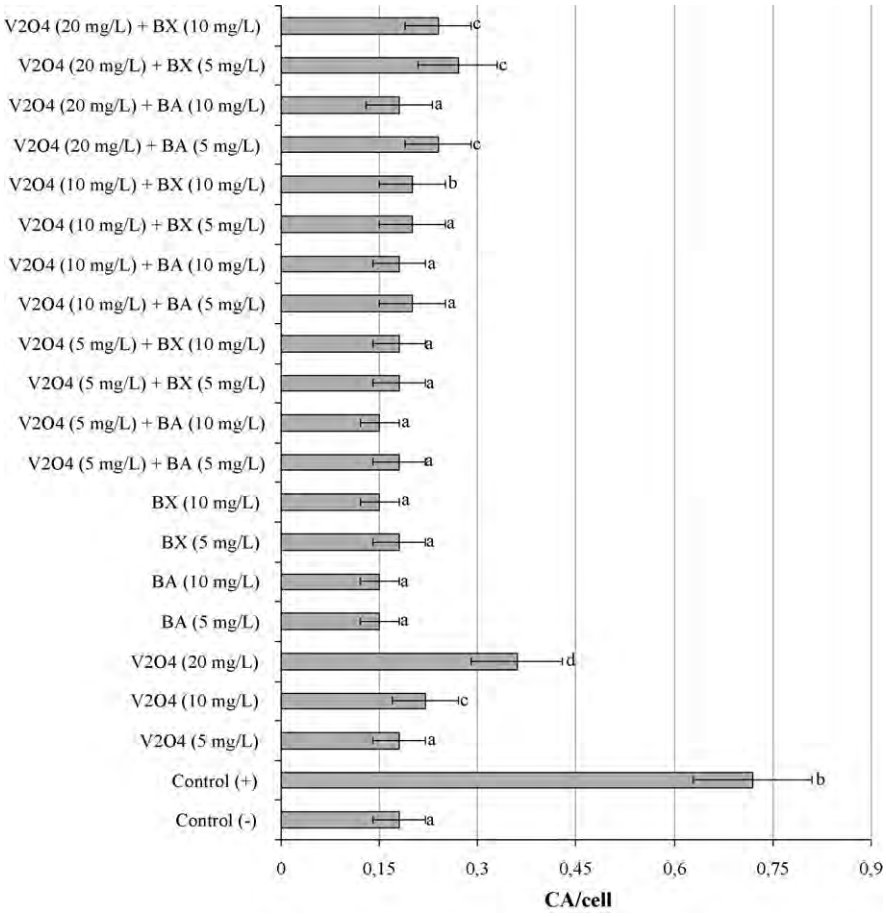


**Fig. 2.** The CA formations in human blood cultures treated with different concentrations of vanadium(IV) tetraoxide and the two boron compounds. Abbreviations are as in Fig. 1.

were put directly on slides using a cytospin, and stained with Giemsa. All slides were coded before scoring. The criteria for scoring MN were as described by Fenech (1993). At least 1000 binucleated lymphocytes were examined per concentration for the presence of one, two or more MN.

### 2.2. Assay of total antioxidant capacity (TAC)

The automated TEAC (Trolox equivalent antioxidant capacity) assay was carried out in isolated erythrocytes with an automated analyzer (Olympus AU 2700, Japan) with commercially available kits (Total Antioxidant Status, Randox Laboratories). In this assay, 2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonate) (ABTS) is incubated for 1 h with metmyoglobin and hydrogen peroxide to produce $ABTS^{-+}$. This species is blue-green. Antioxidants present in the sample cause a reduction in absorption proportional to their concentration (Erel, 2004).

### 2.3. Statistical analysis

Statistical analysis was performed using SPSS software (version 11.5, SPSS, Chicago, IL, USA). The Fisher's least significant difference (LSD) test was used to determine whether any treatment significantly differed from controls or each other. Statistical decisions were made with a significance level of 0.05.

## 3. Results and discussion

Proliferation index is commonly used as a measure of cytotoxicity. The results obtained in the present study show that vanadium(IV) tetraoxide (concentrations above 10 mg/l) induce a reduction in PI in human lymphocytes. These data are similar to the findings from earlier studies (Rodriguez-Mercado et al., 2003). Thus, the data on cytotoxicity for vanadium are not presented. Our previous study reports that the cytotoxicity of boric acid and borax in blood occurs at concentrations above 50 mg/l (Türkez et al., 2007). Similarly, in this study the cultures exposed to boron compounds were found to be sterile at concentrations above 300 mg/l (data not shown). However, it was noteworthy that the high doses of these compounds did not affect the rates of SCEs, CAs and MN.

With respect to the cytogenetic parameters studied in lymphocytes, our results reveal that 5 mg/l vanadium(IV) tetraoxide does not cause significant changes. Whereas, increasing concentrations of vanadium(IV) tetraoxide (10 and 20 mg/l) considerably elevate the frequencies of SCEs and CA formations compared to controls (Figs. 1 and 2).

Our results confirm the clastogenic effects of vanadium(IV) oxidation state both *in vivo* and *in vitro* (Rodriguez-Mercado et al., 2003). As well as vanadium(IV) tetraoxide, vanadates and vanadyl (IV) sulphate compounds also induce SCEs in human lymphocytes (Migliore et al., 1993). On the contrary, *in vitro* exposure of whole blood leukocytes to vanadium pentoxide causes no significant change in SCEs and CAs rates (Roldan and Altamirano, 1990). With knowledge that the chemical species can elicit different toxicities, some authors consider vanadium as a possible carcinogen due to the redox potential presented vanadate (+5 oxidation state) in body fluids and cells is reduced to vanadium(IV), some reports indicate that vanadium(IV) oxidizes a variety of biochemical substrates generating free radicals and producing DNA damage (Altamirano-Lozano et al., 1998).

In present study, the frequencies of MN in blood lymphocytes also exhibit a significant increase after vanadium exposure (Fig. 3).

However, the MN frequency of the negative control was quite low in our investigation. We did not score centric fragments or

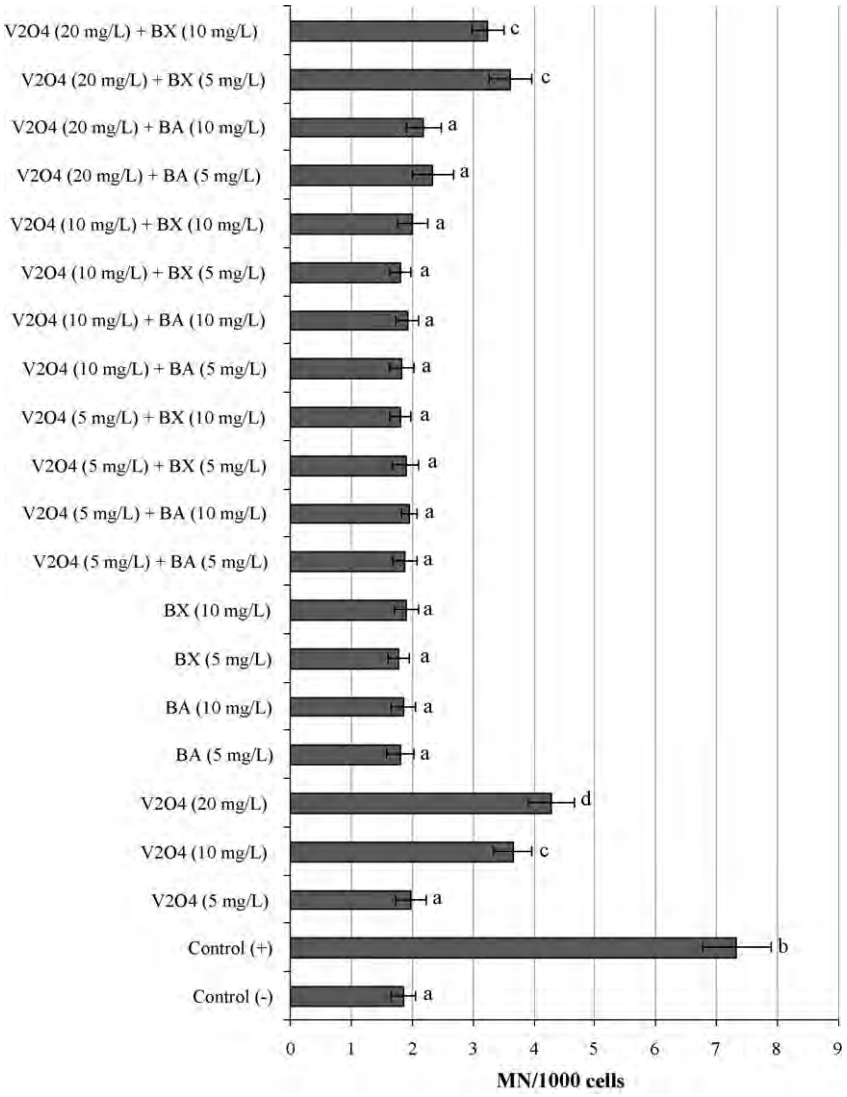


**Fig. 3.** The frequencies of MN (‰) in binucleated cells treated with different concentrations of vanadium(IV) tetraoxide and the two boron compounds. Abbreviations are as in Fig. 1.

internuclear bridges to make our results more reliable. The results of the HUman MicroNucleus (HUMN) project slide-scoring studies have shown that even when laboratories score slides from the same cultures prepared in the same way, there are still large differences between the results of different scorers and laboratories reflecting differences in visual discrimination of the structures scored in the assay and interpretation of scoring criteria (Fenech et al., 2003). And, it has been suggested that this problem can be mitigated by selectively analyzing the frequency of those MN which contain only acentric fragments (Wojcik et al., 2000). The spontaneous frequencies obtained in this study were not discussed with the published data on human lymphocytes in literature. Because the same scoring criteria were performed on all experimental groups including negative controls by an observer. Besides, our volunteers were between the ages of 26 and 28, healthy, non-smoking, and unexposed to any toxic agent occupationally. So the mean values of negative controls were low and quite similar. Several different mechanisms may be involved in the formation of MN, including mitotic spindle disruption (aneugenic) or chromosome breakage (Kirsch-Volders et al., 2000). In mammalian cells, both *in vivo* and *in vitro*, results indicate that vanadium compounds increase rates of MN (Ramirez et al., 1997).

Studies demonstrate that vanadium(IV) has the ability to produce reactive oxygen species (ROS), an event that may play an important role in vanadium toxicity (Zhang et al., 2001) and perhaps in the induction of CA and SCE in human lymphocyte cultures (Rodriguez-Mercado et al., 2003). It is known that ROS are important mediators of damage to cell structures, including lipids and membranes proteins and nucleic acids (termed oxidative stress) (Hooiveld et al., 1998). Oxidative stress increases after exposure to vanadium in human erythrocytes and osteosarcoma cell lines (Abou-Seif, 1998). In addition, vanadyl(IV) compounds cause the oxidation of NADH. The oxidation of NADH is partly associated with the biological toxicity. Thus, NADH-dependent redox cycles play an important role in oxidative DNA damage as depending on the accumulation of vanadium (Kawanishi et al., 2002). However, the harmful effects of ROS and the oxidation of NADH in all reactions are inhibited by the antioxidant activities (Abou-Seif, 1998). A study reveals that boron compounds (until concentrations of 20 mg/l) significantly support the antioxidant capacity in human blood (Türkez et al., 2007). Further support of this suggested locus of action for these compounds is the finding TAC which indicate the response to oxidative stress in the present study (Fig. 4).

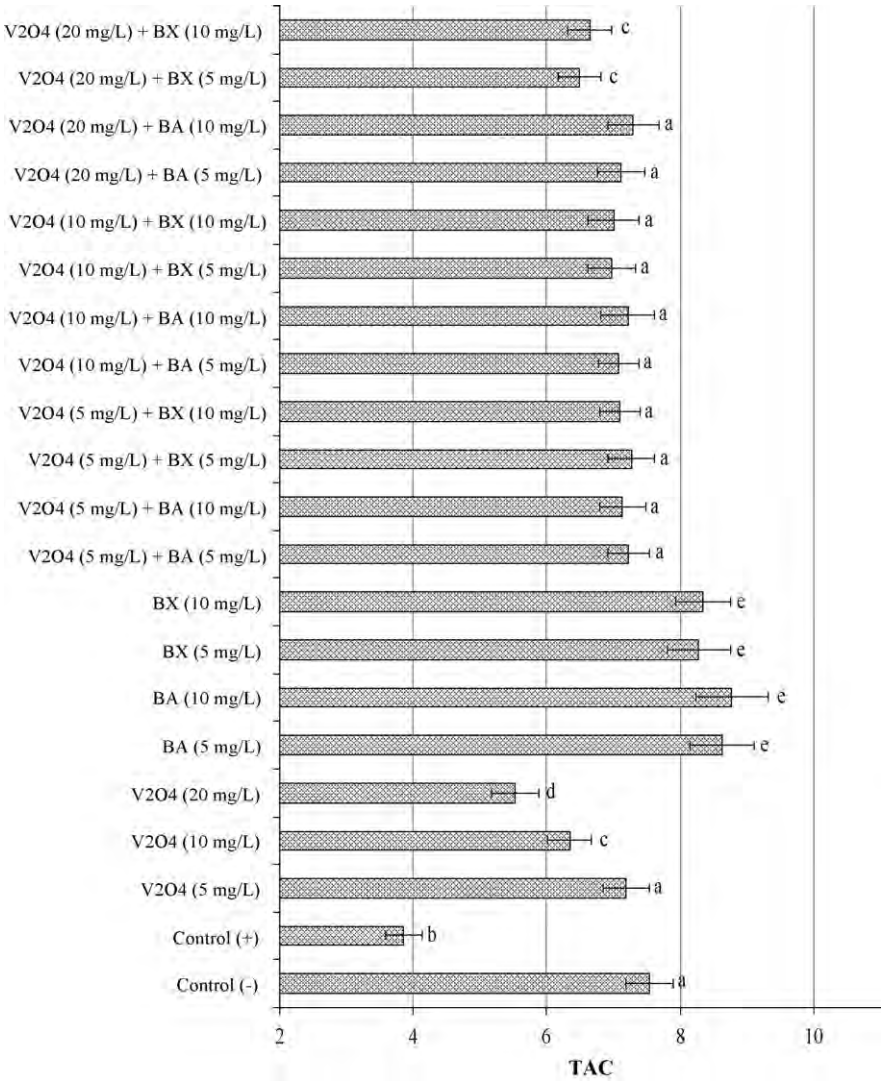


**Fig. 4.** Amount of TAC (μmol/l) capacity in human erythrocytes incubated with vanadium(IV) tetraoxide and the two boron compounds for 1 h. Abbreviations are as in Fig. 1.

Actually, boron compounds apparently decrease the depressive effect of vanadium on TAC. Besides, this study shows that boric acid and borax do not lead to DNA damages in lymphocytes. Moreover, these compounds significantly decrease genotoxic effects of vanadium(IV). However, it is also established that borax could not inhibit the genotoxic effects of vanadium(IV) tetraoxide compared with the control values completely (Figs. 1–3). Very little is known about the biological effects of boron exposure in animal cells (Park et al., 2005). The first hypothesis is that vanadium-induced genetic damage could be prevented by inductive effects of boron compounds on antioxidant capacity in human blood. Although the physiological function of the boron in lymphocytes is unknown, the second one is that boron could also be involved in ion transport (Ma et al., 2003). Recently, *trans*-membrane movement of regulatory cations or anions has emerged as a potential target for cancer therapy in peripheral blood lymphocytes (Yin et al., 2007). Whereas, vanadium has been known to be a potent inhibitor of ion transport in lymphocyte membranes (Sitprija et al., 1993). It is evident that cell volume is directly related to the movement of ions across the plasma membrane and the change of cell volume dramatically induces DNA degradation (Panayioitidis et al., 2006). Again, vanadium makes chemical complexes exhibiting the property of inhibiting or increasing the activity of the enzymes participating in the DNA and RNA synthesis. At this point, the toxic effects of vanadium are also due to the fact of its property of inhibiting many enzymatic systems (Altamirano-Lozano et al., 1998). Thus, SCE and CA may be explained by more than one specific genotoxic effect, such as interference with enzymes involved in cell metabolism (Rodriguez-Mercado et al., 2003). On the other hand, boron is implicated in supporting metabolic activities. Among the effects of boron, that reversible inhibition of serine proteases (SP) or oxidoreductases activities by boron requires pyridine or flavin nucleotides is well known (Hunt, 1998). On the contrary, vanadium forms hydroxyl radicals due to these enzymes in blood cells (Guerrieri et al., 2000; Fickl et al., 2006). However, it is unlikely that any single event can be blamed for protective effects of boron compounds. There is evidence in both *in vitro* and *in vivo* studies that boron has an affinity for hydroxyl groups, and this may be the mechanism that explains the biological effects of boron (Hunt, 1998; Bolanos et al., 2004). Hence, different roles of boric acid and borax against vanadium genotoxicity might have appeared due to their chemical structure and their capacity to form diester bridges between *cis*-hydroxyl-containing molecules.

In conclusion, vanadium(IV) tetraoxide leads to genetic damages depending on dose in human blood. On the contrary, boron compounds can be used safely against vanadium genotoxicity.

Moreover, the anti-genotoxic roles of these compounds are dose-related. Altogether, these results suggest medical and therapeutic usefulness of boric acid and borax for increasing vanadium dosage. Nevertheless, biochemical and physiological studies are needed to explain different anti-genotoxic effects of boron compounds.

## Conflict of interest

None.

## Funding

The authors are also grateful to Gokhan YUKSEL for the linguistic revision of the article. This investigation was supported in part by Ataturk University (BAP-2004-172).

## Acknowledgements

We are grateful to all the volunteers for the blood samples. The author is also grateful to Gokhan YUKSEL for the linguistic revision of the article.

## References

Abou-Seif, M.A., 1998. Oxidative stress of vanadium-mediated oxygen free radical generation stimulated by aluminium on human erythrocytes. Ann. Clin. Biochem. 35, 254.

Altamirano-Lozano, M.A., Roldan-Reyes, E., Rojas, E., 1998. Genetic toxicology of vanadium compounds. In: Nriagu, J. (Ed.), Vanadium in the Environment, Parts I and II. Wiley and Sons, New York, p. 844.

Aragon, M.A., Altamirano-Lozano, M., 2001. Sperm an testicular modifications induced by subchronic treatments with vanadium(IV) in CD-1 mice. Reprod. Toxicol. 15, 145.

Aragon, M.A., Ayala, M.E., Fortoul, T.I., Bizzaro, P., Altamirano-Lozano, M., 2005. Vanadium induced ultrastructural changes and apoptosis in male germ cells. Reprod. Toxicol. 20 (1), 127.

Barreto, R., Kawakita, S., Tsuchiya, J., Mineli, E., Pavasuthipaisit, K., Helmy, A., Marotta, F., 2005. Metal-induced oxidative damage in cultured hepatocytes and hepatic lysosomal fraction: beneficial effect of a curcumin/absinthium compound. Chin. J. Dig. Dis. 6, 31.

Bolanos, L., Lukaszewski, K., Bonilla, I., Blevins, D., 2004. Why boron. Plant Physiol. Biochem. 42 (11), 907.

Devirian, T.A., Volpe, S.L., 2003. The physiological effects of dietary boron. Crit. Rev. Food Sci. Nutr. 43 (2), 219.

Erel, O., 2004. A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation. Clin. Biochem. 37, 277.

Evans, H.J., O'Riordan, M.L., 1975. Human peripheral blood lymphocytes for the analysis of chromosome aberrations in mutagen tests. Mutat. Res. 31, 135.

Fenech, M., 1993. The cytokinesis blocks micronucleus technique. A detailed description on the method and its application to genotoxicity studies in human population. Mutat. Res. 285, 35.

Fenech, M., Chang, W.P., Kirsch-Volders, M., Holland, N., Bonassi, S., Zeiger, E., 2003. HUMN project: detailed description of the scoring criteria for the cytokinesis-block micronucleus assay using isolated human lymphocyte cultures. Mutat. Res. 534 (1–2), 65.

Fickl, H., Theron, A.J., Grimmer, H., Oommen, J., Ramafi, G.J., Steel, H.C., Visser, S.S., Anderson, R., 2006. Vanadium promotes hydroxyl radical formation by activated human neutrophils. Free Radic. Biol. Med. 408 (1), 146.

Guerrieri, N., Scippa, S., Maietti, M., de Vincentiis, M., Cerletti, P., 2000. Protease activity in fractionated blood cells of the vanadium accumulating ascidian *Phallusia mammilata*. Comp. Biochem. Physiol. A Mol. Integr. Physiol. 125 (4), 445.

Hooiveld, M., Heederik, D.J., Kogevinas, M., Boffetta, P., Needham, L.L., Patterson Jr., F D.G., Bueno-de-Mesquita, H.B., 1998. Second follow-up of a Dutch cohort occupationally exposed to phenoxy herbicides, chlorophenols, and contaminants. Am. J. Epidemiol. 147, 891.

Hunt, C.D., 1998. Regulation of enzymatic activity: one possible role of dietary boron in higher animals and humans. Biol. Trace Elem. Res. 66 (1–3), 205.

IPCS (International Program on Chemical Safety), 1985. Environmental health criteria, 46. In: Guidelines for the Study of Genetic Effects in Human Populations. World Health Organisation, Geneva, p. 45.

Kawanishi, S., Hiraku, Y., Murata, M., Oikawa, S., 2002. The role of metals in site-specific DNA damage with reference to carcinogenesis. Free Radic. Med. 32 (9), 822.

Kirsch-Volders, M., Sofuni, T., Aardema, M., Albertini, S., Eastmond, D., Fenech, M., Ishidate, M., Lorge, E., Norppa, H., Surrales, J., von der Hude, W., Wakata, A., 2000. Report from the *in vitro* micronucleus assay working group. Environ. Mol. Mutagen. 35, 167.

Ma, H.T., Venkatachalam, K., Rys-Sikora, K.E., He, L.P., Zheng, F., Gill, D.L., 2003. Modification of phospholipase C-gamma-induced $Ca^{2+}$ signal generation by 2-aminoethoxydipheynl borate. Biochem. J. 376, 667.

Migliore, L., Bocciardi, R., Macri, C., Lo Jacono, F., 1993. Cytogenetic damage induced in human lymphocytes by four vanadium compounds and micronucleus analysis by fluorescence in situ hybridization with a centromeric probe. Mutat. Res. 319, 205.

Migliore, L., Scarpato, R., Falco, P., 1995. The use of fluorescence in situ hybridization with a β-satellite DNA probe for the detection of acrocentric chromosomes in vanadium-induced micronuclei. Cytogenet. Cell. Genet. 69, 215.

Moore, J.A., Expert Scientific Committee, 1997. An assessment of boric acid and borax using the IEHR Evaluative Process for Assessing Human Developmental and Reproductive Toxicity of Agents. Reprod. Toxicol. 11, 123.

Panayioitidis, M.I., Bortner, C.D., Cidlowski, J.A., 2006. On the mechanism of ionic regulation of apoptosis: would the $Na^+/K^+$-ATPase please stand up? Acta Physiol. 187 (1–2), 205.

Park, M., Li, Q., Shcheynikov, N., Muallem, S., Zeng, W., 2005. Borate transport and cell growth and proliferation. Not only in plants. Cell Cycle 4 (1), 24.

Ramirez, P., Easstmond, D.A., Laclette, J.P., Ostrosky-Wegman, P., 1997. Disruption of microtubule assembly and spindle formation as a mechanism for the induction of aneuploidy cells by sodium arsenite and vanadium pentoxide. Mutat. Res. 386, 291.

Rodriguez-Mercado, J., Roldan-Reyes, E., Altamirano-Lozano, M., 2003. Genotoxic effects of vanadium(IV) in human peripheral blood cells. Toxicol. Lett. 144, 359.

Rojas, E., Valverde, M., Altamirano-Lozano, M., Ostrosky-Wegman, P., 1996. Genotoxicity of vanadium pentoxide evaluate by the single cell gel electrophoresis assay in human lymphocytes. Mutat. Res. 359, 77.

Roldan, R.E., Altamirano, L.M.A., 1990. Chromosomal aberrations, sister chromatid exchanges, cell-cycle kinetics and satellite associations in human lymphocyte cultures exposed to vanadium pentoxide. Mutat. Res. 245, 61.

Sakurai, H., 2005. Therapeutic potential of vanadium in treating diabetes mellitus. Clin. Calcium 15, 49.

Sitprija, V., Tungsanga, K., Tosukhowong, P., Leelhahunt, N., Kruerklai, D., Sriboonlue, P., Saew, O., 1993. Metabolic problems in northeastern Thailand: possible role of vanadium. Miner. Electrolyte. Metab. 19 (1), 51.

Türkez, H., Geyikoglu, F., 2006. Protective effects of kernite and probertite against titanium dioxide genotoxicity *in vitro*. In: Güyagüler, T., Karakaş, S., Özdemir, Z., Karakaş, A., Bükülmez, A., Sönmezer, O. (Eds.), Proceedings of 3rd International Boron Symposium, 2–4 November. Ankara, Turkey, p. 475.

Türkez, H., Geyikoğlu, F., Tatar, A., Keleş, S., Özkan, A., 2007. Effects of some boron compounds on peripheral human blood. Z. Naturforsch. C 62, 889.

Valko, M., Morris, H., Cronin, M.T., 2005. Metals, toxicity and oxidative stress. Curr. Med. Chem. 12 (10), 1161.

Villani, P., Cordelli, E., Leopardi, P., Siniscalchi, E., Veschetti, E., Fresegna, A.M., Crebelli, R., 2007. Evolution of genotoxicity of oral exposure to tetravalent vanadium *in vivo*. Toxicol. Lett. 170, 11.

Wojcik, A., Kowalska, M., Bouzyk, E., Buraczewska, I., Kobialko, G., Jarocewicz, N., Szumiel, I., 2000. Validation of the micronucleus-centromere assay for biological dosimetry. Genet. Mol. Biol. 23 (4), 1083.

Yin, W., Cheng, W., Shen, W., Shu, L., Zhao, J., zhang, J., Hua, Z.C., 2007. Impairment of Na(+), K(+)-ATPase in CD95(APO-1)-induced human T-cell leukemia cell apoptosis mediated by glutathione depletion and generation of hydrogen peroxide. Leukemia 21 (11), 2397.

Zhang, Z., Huang, C.S., Li, J.X., Leonard, S.S., Lanciotti, R., Butterworth, L., Shi, X.L., 2001. Vanadate-induced cell growth regulation and the role of reactive oxygen species. Arch. Biochem. Biophys. 392, 311.