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A possible role of oxidative stress in the vanadium-induced cytotoxicity in the MC3T3E1 osteoblast and UMR106 osteosarcoma cell lines

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Abstract

The cytotoxicity and free radical production induced by vanadium compounds were investigated in an osteoblast (MC3T3E1) and an osteosarcoma (UMR106) cell lines in culture. Vanadate induced cell toxicity, reactive oxygen species (ROS) formation and thiobarbituric acid reactive substances (TBARS) increased in a concentration-dependent manner (0.1–10 mM) after 4 h. The concentration–response curve of vanadate-induced cytotoxicity and oxidative stress in MC3T3E1 cells was shifted to the left of the UMR106 curve, suggesting a greater sensitivity of the non-transformed cells in comparison to the osteosarcoma UMR106 cells. Supplementing with vitamin E acetate (80 μM) significantly inhibited ROS and TBARS formation but did not improve the vanadate-dependent decrease in cell number. Other vanadium compounds (vanadyl, pervanadate, and VO/Aspi, a complex of vanadyl(IV) with aspirin) showed different degrees of cell toxicity and induced oxidative stress. Altogether these results suggest that oxidative stress is involved in vanadium induced osteoblastic cytotoxicity, although the mechanism is unknown.

Keywords: Vanadium; Lipid peroxidation; Reactive oxygen species; Cytotoxicity; Osteoblasts; Bone

1. Introduction

Vanadium is a trace element that is widely distributed in the earth. Although vanadium is known to be essential for a number of species, its role as a micronutrient in humans has yet to be established. The main source of vanadium in the

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general population is food and atmospheric vanadium, although their contribution varies between industrial or rural areas (Nriagu, 1998). Vanadium(V) enters the organism by inhalation, the gastrointestinal tract and the skin. Once absorbed, vanadate is reduced to vanadyl(IV) by the glutathione of erythrocytes or by ascorbic acid and other reducing substances in plasma and is transported by albumin and transferrin (Degani et al., 1981). Vanadium is specially stored in certain organs, mainly in bone, kidney and liver (Etcheverry and Cortizo, 1998).

On the other hand, there are now increasing evidence that vanadium can also be toxic to living systems (Domingo, 1996; Sabbioni et al., 1993). An excess of vanadate induces toxic effects in workers of industrial populations possible by oxidative stress (Halliwell and Gutteridge, 1999). As a phosphate analogue, vanadate and its derivatives interfere with a number of enzymes of phosphate metabolism. For instance, it inhibits different ATPases. Vanadate inhibits protein tyrosine phosphatases and therefore resulting in accumulation of phosphorylated protein tyrosine residues in several cells (Gresser and Tracey, 1990). In addition, these properties point the potential capacity of vanadium in the regulation of different physiological processes such as cell growth, differentiation, glucose and lipid metabolism (Stern et al., 1993). Experimental evidence thus suggests that vanadium has insulin- and growth factor-like properties. On the other hand, it has been shown beneficial effects of vanadium compounds in the treatment of experimental diabetic rats (Shechter, 1990) and recently in clinical trials in diabetic patients (Goldfine et al., 1995; Cohen et al., 1995; Halberstam et al., 1996; Cusi et al., 1997). These facts suggest that vanadium compounds could be potential pharmaceutical tools to ameliorate certain diseases. To understand the insulin-like properties and the toxicity of vanadium compounds it will be essential to know the mechanism of action as well as the susceptibility of different cells and tissues to the effects of vanadium.

It was shown early that vanadate induces lipid peroxidation in isolated hepatocytes as well as in the perfused rat liver (Stacey and Klaassen, 1981; Younes and Strubelt, 1991a). The role of oxidative mechanisms in vanadate-induced hepatotoxicity was suggested by the use of antioxidant agents (Younes et al., 1991b). Recently, Krejsa et al. (1997) has suggested a role for oxidative stress in the action of vanadium on the lymphocyte signal transduction pathway. These authors have shown that redox-dependent effects occur in concert with redox-independent actions.

Using a model of murine osteoblast-like cells in culture, we have previously shown that vanadium compounds exert a biphasic effect on cell growth: low micromolar concentrations stimulated cell proliferation while high concentrations inhibit cell proliferation (Cortizo and Etcheverry, 1995; Etcheverry et al., 1997; Barrio et al., 1997; Sálice et al., 1999). In addition, we have observed that UMR106, an osteosarcoma cell line, was less affected by high levels of vanadium (Cortizo and Etcheverry, 1995), while non-transformed MC3T3E1 mouse calvaria osteoblastic cells were very sensitive to the growth inhibitory effects of this element (Sálice et al., 1999). Moreover, the MC3T3E1 line showed morphological alterations after the incubation with vanadium derivatives (Etcheverry et al., 1997; Sálice et al., 1999).

The aim of the present study was to investigate if oxidative mechanisms are involved in the cytotoxic effects induced by vanadium compounds in osteoblast-like cells. For this purpose, we determined: (1) osteoblastic vanadium-induced cytotoxicity by the crystal violet bioassay, a determination that evaluates cell survival; (2) lipid peroxidation estimated by thiobarbituric acid reactive substances (TBARS) and the formation of reactive oxygen species (ROS) using the probe dihydrorhodamine123 (DHR); and (3) possible prevention of cytotoxic and oxidative stress by the preincubation with vitamin E.

2. Materials and methods

2.1. Materials

Vanadium (IV) oxide sulfate (vanadyl sulfate) was obtained from Merck (Darmstadt, Germany). Sodium orthovanadate (V), 2-thiobarbituric acid,

1,1,3,3-tetramethoxypropane, and rhodamine123 were purchased from Sigma Chemical Co. (St. Louis, MO). Dihydrorhodamine123 (DHR) was from Molecular Probes, (Eugene, OR). Vitamin E was a gift from RP Scherer Argentina S.A.I.C (Buenos Aires, Argentina). Tissue culture material was provided by Corning (Princeton, NJ). Dulbecco's Modified Eagles Medium (DMEM) and trypsin-EDTA were supplied by Gibco (Gaithersburg, MD) and fetal bovine serum (FBS) from Gen (Buenos Aires, Argentina). All other chemicals used were of analytical grade from Sigma.

2.2. Solutions

Fresh stock solutions of vanadyl (IV) sulfate (VO) and sodium o-vanadate (V) were prepared in distilled water at a 100 mM concentration and diluted according to the concentrations indicated in the legends of the figures (Cortizo and Etcheverry, 1995). Peroxovanadate (perV) was prepared according to Trudel et al. (1991) where stock solutions containing 1 mM orthovanadate and 2 mM H_2O_2 were incubated for 15 min at room temperature and then treated with catalase (200 μg/ml) for 3 min before immediate use in experiments, after appropriate dilutions. A complex of vanadyl with aspirin (VO/Aspi) was synthesized and characterized as we have described (Etcheverry et al., 1999).

2.3. Fluorescent probes

DHR was prepared as a 25 mM and rhodamine123 as a 0.5 mM stock solutions in nitrogen-purged dimethylformamide and stored in the dark at −20°C until used (Royall and Ischiropoulos, 1993). A 10 mM stock solution of DHR was prepared fresh each day in Hanks' balanced salt solution (HBSS). A standard curve of rhodamine123 was constructed from 0.1 to 5 μM.

2.4. Cell culture and incubations

MC3T3E1 osteoblastic mouse calvaria-derived cells and UMR106 rat osteosarcoma-derived cells were grown in 75 cm^2 flasks at 37°C in a humidified 5% CO_2 atmosphere in DMEM supplemented with 100 U/ml penicillin, 100 μg/ml streptomycin and 10% (v/v) FBS. When 70–80% confluence was reached, cells were subcultured using 0.1% trypsin-1 mM EDTA in Ca^{2+}–Mg^{2+} free phosphate buffered saline PBS (Cortizo and Etcheverry, 1995; Etcheverry et al., 1997). For experiments, cells were seeded in six- or 24-well plates at a density of 2.5×10^4 cells/well. When cells reached confluence, the monolayers were washed with HBSS and incubated as describe below. The effect of the antioxidant was tested by incubating cells with 80 μM vitamin E in DMEM without FBS for 24 h at 37°C. At this concentration of vitamin E, a maximal inhibition of TBARS formation was obtained without toxic effects. Control cells were preincubated in the absence of the vitamin supplement but in the presence of vehicle (0.1% ethanol).

2.5. Cell survival. Crystal violet bioassay

The viability of osteoblast-like cells was determined by the crystal violet bioassay (Okajima et al., 1992). Briefly, cells were washed with PBS and fixed with 5% glutaraldehyde for 10 min. The cultures were then stained with 0.5% crystal violet in 25% methanol for 10 min. Therefore, the excess dye was discarded and the plates were washed with water and dried. The dye taken up by the cells was extracted using 0.1 M glycine/HCl buffer, pH 3.0/30% methanol and transferred to test tube. Absorbance was read at 540 nm after a suitable dilution. We have previously shown that under these conditions, the colorimetric bioassay strongly correlated with viable cell number measured by cell counting in a Neubauer chamber ($r = 0.90$, $P < 0.001$; $r = 0.97$, $P < 0.001$; for UMR and MC3T3E1 cells, respectively) (Cortizo and Etcheverry, 1995; Sálice et al., 1999).

2.6. Determination of ROS formation

Intracellular ROS generation in osteoblast-like cells was measured by oxidation of DHR to rhodamine. This probe measures levels of ROS intermediates peroxynitrite and the hydroxyl radical $OH^{\bullet}$ but not $O_2^{\dot{}-}$ or H_2O_2 (Royall and Ischiropoulos, 1993). Briefly, osteoblast-like cells were

incubated at 37°C in 1.5 ml HBSS alone (basal condition) or with several vanadium compounds, in the presence of 10 μM DHR (Krejsa et al., 1997). Media were separated and the cell monolayers rinsed with PBS and scraped into 1 ml 0.1% Triton-X100. The cell extracts were then analyzed for the oxidized product rhodamine by measuring fluorescence (excitation wavelengh, 495 nm; emission wavelengh, 532 nm), using an Aminco-Bowman SPF100 spectrofluorometer equipped with a Hamamatsu R928 photomultiplier tube. Results were corrected for protein content, which was assessed by the method of Bradford (1976).

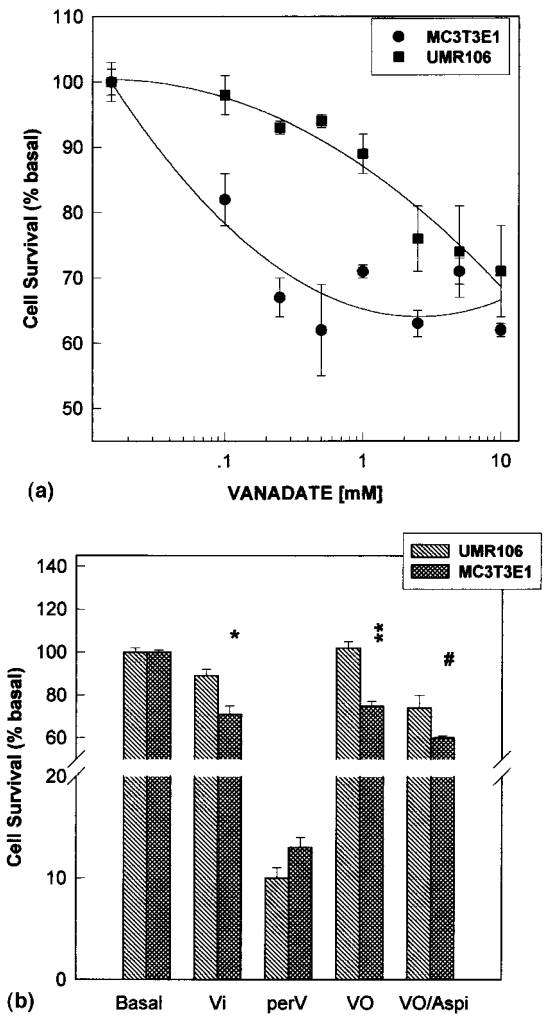

Fig. 1. Cell survival of osteoblast-like cells after a 4-h incubation with vanadate (A) or 1 mM of various vanadium compounds (B). Values are expressed as % basal survival and represent the means ± S.E.M., $n = 6$. Statistically significant differences between the effect of each compound in both cell lines: # $P < 0.05$, * $P < 0.01$, ** $P < 0.001$.

2.7. TBARS production

Cells were incubated in 1.5 ml HBSS without (basal condition) or with several vanadium compounds at 37°C for different incubation periods. Media were separated, cells washed with PBS and scraped in 200 μl 0.1% Triton-X100. An aliquot was saved for protein determination. To measure the extent of lipid peroxidation, the TBARS assay was carried out as reported by Ohkawa et al. (1979). Each cell extract was mixed with 1 ml 20% acetic acid pH 3.5 and 1 ml 0.78% aqueous solution of thiobarbituric acid. After heating at 95°C for 1 h, the mixtures were centrifuged at 3000 rpm for 15 min and the red pigment in the suppernatant fractions were estimated by absorbance at 532 nm. The lipid peroxide levels were expressed in terms of nmol of malondialdehyde per mg protein, using 1,1,3,3-tetramethoxypropane as standard.

2.8. Statistical analysis

At least three experiments were performed for each experimental condition. Results are expressed as mean ± S.E.M. Statistical differences were analyzed using Student's *t*-test. Correlation among different parameters was analyzed using Pearson's correlation coefficient as well as the linear regression method.

3. Results

3.1. Vanadate induces cytotoxicity in osteoblast-like cells

The cytotoxicity induced by vanadate was estimated measuring cell survival in two lines of murine osteoblast-like cells by the crystal violet bioassay. Fig. 1A shows that in the presence of low concentrations of vanadate (0.1–0.5 mM) cell survival was preserved in the UMR106 cells. Nev-

ertheless, increasing the concentration of vanadate up to 10 mM caused a concentration-dependent decrease in cell survival, which was statistically significant after 1 mM. In the MC3T3E1 cells, vanadate caused a greater cytotoxic effect, significantly decreasing cell number at 0.1 mM, with a concentration–response curve to left of the UMR106 cell curve. This observation suggests a greater sensitivity to vanadate cytotoxicity for the non-transformed line. In order to evaluate further this differential effect, other vanadium compounds were tested for their ability to induce loss of viable cells (Fig. 1B). After a 4 h incubation of UMR106 cells with 1 mM of different vanadium compounds, perV and VO/Aspi were comparatively more toxic than vanadate, while VO did not affect cell survival. In the non-transformed MC3T3E1 cell line, perV was also the more toxic compound, followed by VO/Aspi. Vanadate and vanadyl exerted a comparable effect on these cells. No toxic effect was observed after incubation with 1 mM H_2O_2 or aspirin in MC3T3E1 or UMR106 cells (data not shown). Altogether, these results show a similar trend in the potency of the four vanadium derivatives tested in both cell lines (perV $\gg$ VO/Aspi $>$ Vi $\geq$ VO), but with a greater toxic effect for most of the compounds in the MC3T3E1 cells.

3.2. Detection of intracellular ROS

Osteoblast-like cells were loaded for 4 h with DHR and the oxidized content of the fluoresecent product rhodamine was assessed. The basal content of rhodamine produced was four-fold higher than in the non-transformed osteoblastic line. Fig. 2A shows that vanadate caused an incremental increase in the ROS formation after 4 h of incubation in both cell lines, with a threshold of 0.1 mM ($P < 0.001$) and a saturation at approximately 2.5 mM. Furthermore, the UMR106 osteosarcoma line is more resistant than MC3T3E1 cells to the vanadate-induced free radical formation. In experiments with UMR106 cells, the dose–response curve was displaced to the right of the MC3T3E1 curve, suggesting lower sensitivity. The ROS production was also time-dependent (Fig. 2B), reaching a maximum after 3–4 h of incubation and detected as early as 30 min, in the presence of 2.5 mM vanadate.

Inductional ROS formation was also evaluated using other vanadium derivatives (Fig. 2C). The complex with aspirin generated minor although significant free radicals in both cell lines. Pervanadate was equivalent to VO/Aspi in the osteosarcoma cells but as potent as VO in the non-transformed MC3T3E1 line. For most of the compounds, the MC3T3E1 cells were more sensitive than UMR106 cells.

3.3. TBARS production during vanadate cytotoxicity

Lipid peroxidation of vanadate-treated osteoblasts as well as untreated (basal) cells was evaluated after a 4 h incubation. The basal TBARS production was 4.1-fold higher in untreated UMR106 than in MC3T3E1 cultures. The TBARS increased with the vanadate concentration in the media in a concentration-dependent manner (Fig. 3A), reaching a maximum at 5 mM. At low concentrations (0.25 and 0.5 mM) a higher sensitivity of the MC3T3E1 cells toward the vanadium-induced TBARS formation was observed. The time-dependent increase in 2.5 mM vanadate-induced TBARS production in MC3T3E1 cells is shown in Fig. 3B, and can be seen to have reached a maximum after 3–4 h of incubation, in parallel to the kinetics of ROS generation (Fig. 2B).

Different vanadium compounds were evaluated at 1 mM concentrations (Fig. 3C). In these experiments, VO and VO/Aspi were the more potent TBARS-inductors in both cell lines. Moreover, the only significant difference between the two lines was observed in the case of VO/Aspi.

3.4. Effect of vitamin E on vanadate toxicity

The cytotoxicity and oxidative stress induced by vanadate on MC3T3E1 osteoblast-like cells were further evaluated after 24 h of 80 μM vitamin E supplementation in the culture media. This concentration was previously shown to do not induce toxicity in the MC3T3E1 cells (data not shown).

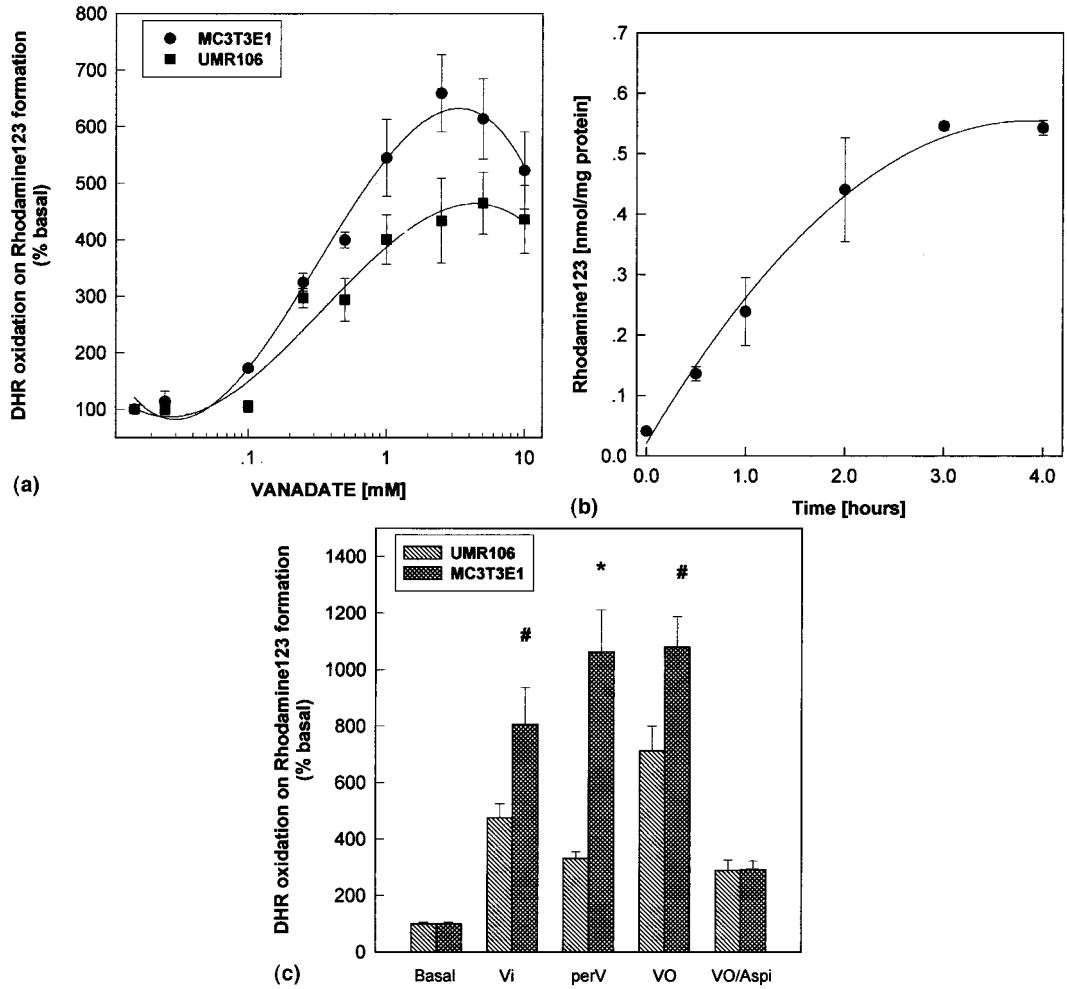

Fig. 2. Effect of vanadate on DHR oxidation to rhodamine. Osteoblast-like cells were incubated with: (A) 10 μM DHR and different concentrations of vanadate for 4 h; (B) presence of 2.5 mM vanadate for several incubation periods (MC3T3E1); or (C) 1 mM of different vanadium compounds for 4 h. The generation of ROS was evaluated as described in Section 2. Values represent the means ± S.E.M., $n = 8$. Statistically significant differences between vanadium-induced ROS in both cell lines are: # $P < 0.05$, * $P < 0.01$.

Table 1 shows that no protection was exerted by vitamin E upon the toxicity of vanadate, as evaluated by cellular survival. The vanadate-induced DHR oxidation was significantly prevented by the vitamin E. This antioxidant blunted about half of the vanadate-induced ROS generation when 1 or 2.5 mM vanadate was tested.

The osteoblast content of TBARS produced by similar concentrations of vanadate was also significantly inhibited by the preincubation of cells with vitamin E (Table 1). No effect on lipid peroxidation was observed when MC3T3E1 cells were incubated with vitamin E alone, suggesting no toxicity at the concentration used in this study.

4. Discussion

In this study it was observed that vanadate, depending on the concentration, causes cytotoxic effects in osteoblast-like cells. This was evidenced by the crystal violet bioassay (Fig. 1A). Low concentrations of vanadate did not show any effect on cell number. However, high doses of

vanadate induced loss of cells; the concentration–response curve of vanadate-induced cytotoxicity in MC3T3E1 cells was shifted to the left of the UMR106 curve, suggesting a greater sensitivity of the non-transformed cells in comparison to the osteosarcoma UMR106 cells. This observation is in agreement with our previous results of vanadate-induced cell growth inhibition in both cell lines incubated 24 h with low concentrations (25–100 μM) of different vanadium compounds (Cortizo and Etcheverry, 1995; Sálice et al., 1999). In the present study we evaluated the acute effect of vanadium on the oxidative stress and cell survival in the same model of osteoblast-like cells. Previous studies have shown that vanadate(V) and vanadyl(IV) can cause oxidative stress through the formation of TBARS and hydroxyl free radicals in different cell types, incubated for short periods (Stacey and Klaassen, 1981; Younes and Strubelt, 1991a; Bay et al., 1997). These effects were associated with cellular death or membrane injury. The results obtained in our model system suggest that the vanadium-induced cytotoxicity is partially dependent on oxidative stress. This pro-

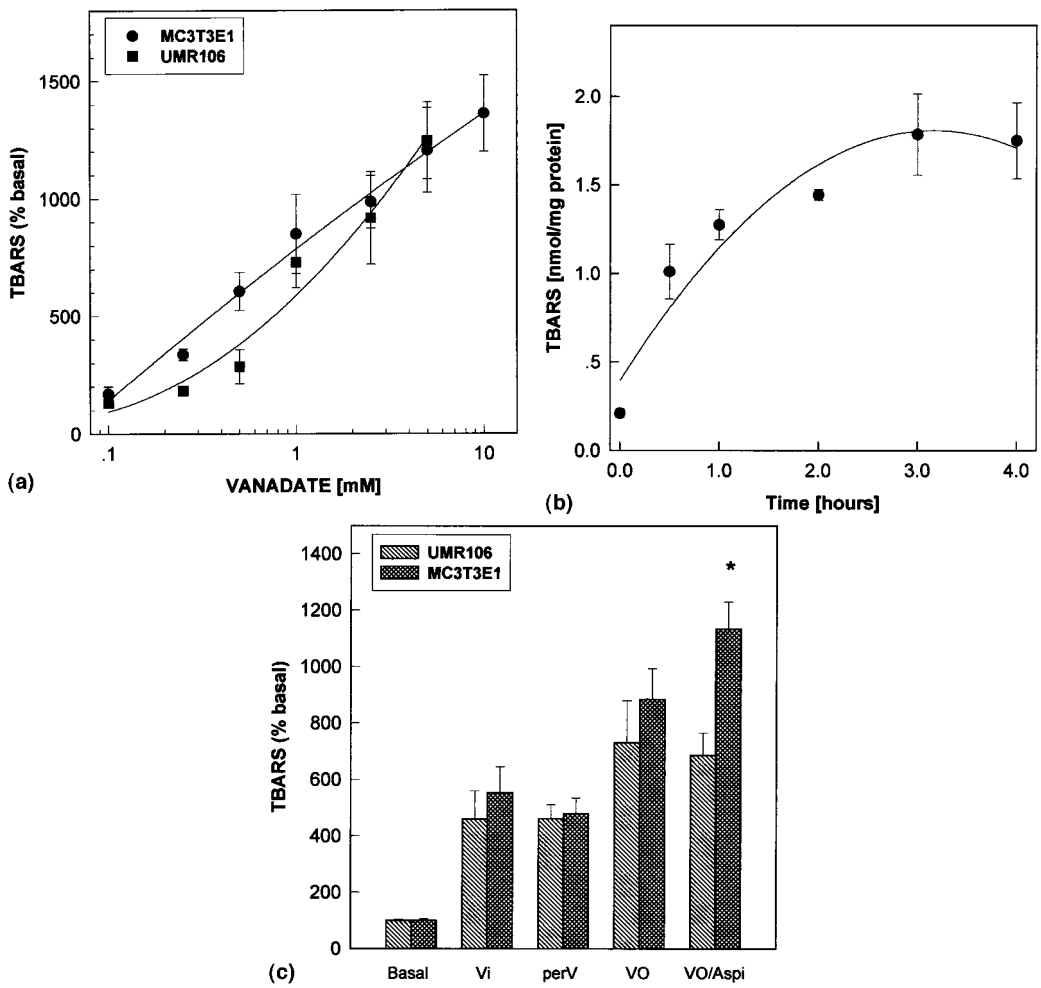

Fig. 3. Effect of vanadate on lipid peroxidation as evaluated by TBARS production. Cells were incubated with: (A) various vanadate concentrations for 4 h; (B) 2.5 mM vanadate for different incubation periods (MC3T3E1); or (C) 1 mM of various vanadium complexes for 4 h. The cellular TBARS were evaluated using a MDA standard. Values represent the means ± S.E.M., $n = 8$. Statistically significant differences between each compound in both cells lines are: * $P < 0.01$.

Table 1
Effect of vitamin E on vanadate-induced cytotoxicity, DHR oxidation and TBARS formation in MC3T3E1 osteoblast-like cells[a]

Vanadate (mM)	Vitamin E (80 μM)	Cell survival (% basal)	Rhodamine123 (nmol/mg protein)	TBARS (nmol MDA/mg protein)
0	−	100 ± 3	0.034 ± 0.007	5.70 ± 0.62
	+	96 ± 3	0.045 ± 0.011	4.84 ± 0.31
1	−	66 ± 4	0.280 ± 0.030*	13.80 ± 1.01*
	+	64 ± 3	0.150 ± 0.040*	8.98 ± 0.82*
2.5	−	31 ± 1	0.330 ± 0.040*	15.70 ± 0.95*
	+	29 ± 4	0.180 ± 0.040*	10.50 ± 0.76*

[a] Values given are means ± S.E.M., $n = 4$. Statistically significant differences are obtained in comparison with the basal condition (without vanadate or vitamin E).
* $P < 0.05$.

posal is supported by the following evidence: vanadium induced a cytotoxic effect as well as an oxidative stress in a concentration-dependent manner (Fig. 1A, Fig. 2A and Fig. 3A). The oxidative stress caused by vanadium in both osteoblast-like cells was evaluated by determining the rhodamine123 fluorescence and TBARS production as parameters related to the generation of reactive oxygen species. Both parameters were increased by vanadate in a time-dependent manner, with a maximum effect after 3 h of incubation.

MC3T3E1 cells were more sensitive than UMR106 to vanadate cytotoxicity, as well as to the induction of oxidative stress (TBARS and ROS) (Fig. 2A and Fig. 3A), suggesting a role for the pro-oxidative effect of vanadate in its toxicity. A statistically significant negative correlation was found between cell survival and the TBARS formation ($P < 0.02$). Furthermore, TBARS measurements showed a positive correlation with DHR oxidation ($P < 0.01$).

Other investigators have shown lipid peroxidation and cell damage in liver tissue that suggests a causative role of the pro-oxidant activity of vanadate in hepatic cytotoxicity (Younes and Strubelt, 1991a). In the osteoblatic cell culture system used in this study, vanadate induced lipid peroxidation, as evaluated by TBARS formation. This effect was time and concentration dependent (Fig. 3A and B). At low concentrations, MC3T3E1 cells were more sensitive to vanadate than UMR106 cells. However, higher concentrations (> 2.5 mM) were equally and markedly toxic for both cell lines. At these concentrations, similar number of cells survived after 4 h of incubation with vanadate in both lines (Fig. 1A). All presented evidence in this study is suggestive of a more deleterious effect of vanadate on cell survival in the non-transformed MC3T3E1 line, associated with a higher ROS generation and lipid peroxidation.

The use of different vanadium compounds has allowed us to further investigate the relationship between cell toxicity and oxidative stress caused by vanadium derivatives. We assessed the cytotoxic effects of vanadium using different derivatives of vanadium (IV) and (V). Pervanadate was the most potent inductor of cell toxicity in both cells lines, while VO was the weaker agent. On the other hand, VO/Aspi, caused stronger cell toxicity than Vi in both osteoblastic lines. These results suggest that the oxidation state of vanadium is not the only factor that plays a role in the cytotoxicity in either MC3T3E1 and UMR106 cells. For instance, VO/Aspi, a vanadium(IV) compound was more toxic than Vi, a vanadium(V) derivative. On the other hand, the nature of vanadium ligands seems to have an important influence in this aspect, as can be seen in the case of the peroxovanadium compound.

The vanadium is an element that exists in several oxidation states. In the presence of different cellular systems it may undergoes redox cycling.

Through this mechanism $O_2^{\bullet -}$ as well as $OH^{\bullet}$, and lipid peroxides could be generated and consequently, oxidative damage can be promoted. Different vanadium compounds could enter into the redox cycling with different abilities. In addition, other factors such as the uptake and compartmentation may determine the different toxicity of various vanadium compounds.

The ROS formation enhanced by various vanadium compounds suggests that this effect, as evaluated through DHR oxidation, was dependent on the species of vanadium and the cellular type (Fig. 2C). For most of the compounds, with the exception of VO/Aspi complex, MC3T3E1 cells seem to be more sensitive to oxidative stress than UMR106 cells. This effect was not completely dependent on the oxidation state of vanadium compounds, since vanadyl(IV), pervanadate and vanadate(V) were equally potent in the non-transformed cell line. On the other hand, VO and vanadate show stronger effects than VO/Aspi and perV in the osteosarcoma cell line. Only two reports have evaluated the free radical production induced by vanadium compounds using similar methods to the DHR oxidation. In one of these studies, treatment of lymphocytes for 2 h with vanadate, BMOV (a vanadium(IV) complex with maltol) or pervanadate, differentially induced oxidative stress, pervanadate being the more potent derivative (Krejsa et al., 1997). In liver cells, vanadyl(IV) induced oxidative stress via generation of hydroxyl free radicals (Bay et al., 1997). Altogether, the experimental evidence seems to indicate that sensitivity to oxidative stress by vanadium compounds is dependent on cell type and geometry requirements.

The level of free radicals inside the cells is a product of a balance between the generation of these radicals and the antioxidant defences in the cells. For instance, the depletion of GSH levels in the presence of vanadate has been reported in perfused liver by Younes and Strubelt (1991a). A similar effect was described in liver cells incubated with vanadyl(IV) (Bay et al., 1997). Thus, the depletion of GSH as well as other reducing substances, or alterations in detoxifying enzymes, could lead to an increased susceptibility of the cells to vanadium-induced toxicity.

The comparisons of TBARS formation in both cell lines incubated with the different vanadium compounds (Fig. 3C) did not confirm our previous observation that MC3T3E1 cells are more sensitive than UMR106 cells to oxidative stress and cytotoxicity. Although higher TBARS values were found in the non-transformed cells, the differences between the cell lines were not statistically significant, with the sole exception of VO/Aspi ($P < 0.01$). This seems to indicate that lipid peroxidation is not the only factor responsible for the higher sensitivity of MC3T3E1 cells to the vanadium-induced toxicity. A different level in the antioxidant content may explain its sensitivity.

Vitamin E, acting as a potent peroxyl radical scavenger, inhibits the free radical chain reaction of lipid peroxidation (Van Acker et al., 1993). The antioxidant effects of this vitamin have been extensively described (Mabile et al., 1995; Rego et al., 1998). The results obtained with this antioxidant suggest that the vitamin only partially, although significantly, inhibits oxidative stress in osteoblast-like cells incubated with vanadate. However, the cytotoxicity was not prevented. In agreement with our results, in a perfused liver system, other antioxidants, also protected the tissue against lipid peroxidation but only partially prevented the injury initiated by vanadate (Younes et al., 1991b). These results suggest that the vanadium derivative-induced cell toxicity cannot be simply accounted for their ability to produce oxidative stress. Furthermore, other factors could be involved in the lack of effect of vitamin E to prevent vanadate-induced toxicity. The uptake, compartmentation and distribution of vitamin E within the cell may exert significant effects in relation to cytotoxicity.

In conclusion, vanadium-induced cytotoxocity is a complex phenomenon depending on several factors. An increase in oxidative stress is involved in the toxic effect of vanadium in osteoblastic cells. In addition, the phenotypic characteristics of the cells, as well as their mechanisms of defence and certain geometric features of the vanadium species, also play a role in vanadium-induced cytotoxicity.

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