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Ultrastructural Nuclear Changes in Mice Spleen Lymphocytes after Vanadium Inhalation

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Abstract

Vanadium (V) is an air pollutant, which results from burning of fossil fuels, liberated as vanadium oxides, which are the most toxic compounds. Spleen is a vanadium target organ, and scarce research of the effects of vanadium and the immune system has been published. We previously report histologic modifications in the spleen in a mice inhalation model characterized by a very large and non-clearly delimited germinal centers size, as well as an increased CD19+ cells and a decline in antibody production after immunization. Since nuclear integrity is the support for genetic expression and cell cycle control, we decided to explore, in our model ultrastructural nuclear changes. Male mice were exposed to V205 (0.02M) for three months, twice a week by inhalation Spleen was obtained and processed for TEM. We observed striking nuclear changes such as: invaginations, blebs, multilobulations, and chromatin redistribution, pseudoinclusions, increased nucleolar size and number. All these changes suggest a direct interaction of vanadium with nucleoskeleton as it has been reported for its cytoplasmic counterpart. These alterations in nuclear structure could endorse neoplastic changes, which will need further evaluation.

Keywords: Vanadium; Lymphocytes; Ultrastructure nuclear changes

Introduction

Vanadium is an air pollutant emitted as V205 during fuel combustion. An increase in vanadium (V) concentration in the atmosphere has been reported [1,2] mainly in countries like Venezuela and Mexico due to high V concentrations in their fuel, which are reflected in higher concentrations in air suspended particles emitted after fuel burning [3].

Reports about V toxic effects in different organs such as: testes [4,5], kidney, liver [6], nervous system [7,8] and recently the spleen [9] have increased our knowledge about this element. However, limited information is accessible in the literature about V effect on the immune system.

The spleen, an encapsulated organ, is responsible for the immune secondary response and for filtering blood; V accumulates in this organ, and produces toxic effects. In a previous report of our group, we observed splenomegaly, vanadium produces loss of the relationship between red and white pulp, as a result of the increase in the size of the white pulp, and very large non-clearly delimited germinal centers, as well as an increase in CD19+ cells [9,10]. Additionally, we observed changes in nuclear morphology in peripheral blood lymphocytes and lymphoid organs such as thymus and spleen, however these have not been characterized and the mechanism by which vanadium may induce these changes has not been investigated.

The nucleus is membranous organelle in which the control of diverse activities such as replication, DNA transcription and genetic expression regulation. Nuclear morphologic modifications have been reported in several pathologies, mainly in lymphomas and other neoplastic diseases [11].

Some metals are considered carcinogenic for humans and animals [12,13]. This characteristic is also shared by vanadium, because of its chemical atributes. The nuclear alterations observed in our model, persuade us to carry on a detailed ultrastructural analysis of nuclear changes in lymphocytes from the spleen after vanadium exposure.

Methods

Animals

CD-1 male mice weighing 35g were housed in hanging plastic cages under controlled light conditions (12 h light/12 h dark regime) and fed with Purina rat chow and water ad libitum. The experimental protocol was in accordance with the Animal Act of 1986 for Scientific Procedures. Inhalation exposures were performed as described by Fortoul et al. [9]. Briefly, seventy-two animals were placed randomly in acrylic chambers connected to an ultra nebulizer (UltraNeb99 Devilbiss, Somerset, PA, USA) Inhaling 0.02M V2O5 (Sigma, St. Louis, MO, USA) 1 h twice a week. Particle size was less than 5μm [14]. Control mice inhaled only the vehicle, saline, for the same period. Three exposed animals and three controls were sacrificed weekly, from one to 12 weeks during vanadium pentoxide inhalation. Animals were anesthetized with i.p. sodium pentobarbital and perfused via aorta with saline containing 2% glutaraldehyde and 2% paraformaldehyde in 0.1M phosphate buffer. Spleen was removed, and placed in fixative solution for two hours and processed for its analysis in a Zeiss EM-10 Electron Microscope (TEM). From each exposure time, three exposed and three control mice were sacrificed beginning at first week and ending at the twelve.

Electron transmission microscopy

The tissue blocks were washed with cacodylate buffer and postfixed in 1% osmium tetroxide for 2 hours. After this, they were dehydrated in an ethanol series, and embedded in Araldite 6005. To locate suitable areas, semithin sections of 0.5 μm thickness were sectioned and stained

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with 2% toluidine blue. After examination of the toluidine blue-stained sections, ultrathin sections (60-80 nm thickness) were cut and double-stained with uranyl acetate and lead citrate. All sections were observed under a transmission electron microscope and photographed with a Zeiss EM-10 TEM [14].

Morphological and statistical analysis

From each individual fifteen fields were randomly selected and evaluated in a 55μm^2 area. In each field, the total cells were counted, as well as the cells with nuclear modifications, in order to obtain the percentage of cells with nuclear alterations. A total of 450 cells from each individual were analyzed, and the nuclear changes evaluated were: lobulations, invaginations or evaginations, swelling, modifications in the chromatin pattern distribution and pseudoinclusions in accordance with literature [11,15,16]. Percentages of each alteration were calculated. ANOVA with Tukey's post-test was performed to identify statistical significance for the changes observed compared with controls ($p<0.05$).

Results

Nuclear morphology

Control animals exhibited lymphocytes with a well-delimited round nucleus, heterochromatin distribution displaced to the nuclear envelope. The nuclear membrane was well delimited. In addition, one nucleolus was identified (Figure 1A).

In treated subjects, since the first week during the exposure, changes Such as lobulations (Figure 1B), invaginations (Figure 1C) and deep evaginations (Figure 2E), in close relation to very evident chromatin redistribution, with increased heterochromatin (Figures 1B-D) were observed. In addition, pronounced perinuclear cisterns (Figure 2 B), with increased number and size of nuclear pores polarized toward the lobulations (Figures 1D and 2A), differentiated exposed lymphocytes from controls. Because of the nuclear membrane folding, pseudoinclusions were observed. In some case, cytoplasmic organelles seemed to be located in the nucleus (Figure 2C). In addition, more and larger nucleolus distinguished the exposed lymphocytes (Figure 2 D).

Quantification of nuclear changes

During the observation in the Transmission Electron Microscope (TEM), the percentage of altered lymphocytes with nuclear changes was calculated comparing controls versus exposed mice. In controls the percentage of changes was low (0.9%) (Figure 3). While in the exposed, it was higher (37.5%). Modifications in nuclear morphology were observed since the first week of the exposure and increased during the experiment (twelve weeks) (ANOVA, Tukey's $p<0.001$) (Figure 3).

Discussion

Industrial development has increased the release into the atmosphere of thousands of xenobiotics such as metals, including vanadium. This transition element interacts with DNA and a wide variety of proteins including those related with cellular cycle, cellular death and cytoskeleton. These interactions could explain its neoplastic affect, which nowadays is controversial.

In this report, we identify for the first time the ultraestructure nuclear changes in spleen lymphocytes after vanadium inhalation, which are similar to those exhibited in malignant cells. These changes and those reported by Piñon-Zarate et al. [10] suggest lymphocyte activation and its proliferation, which together could result in a lymphoproliferative disorder.

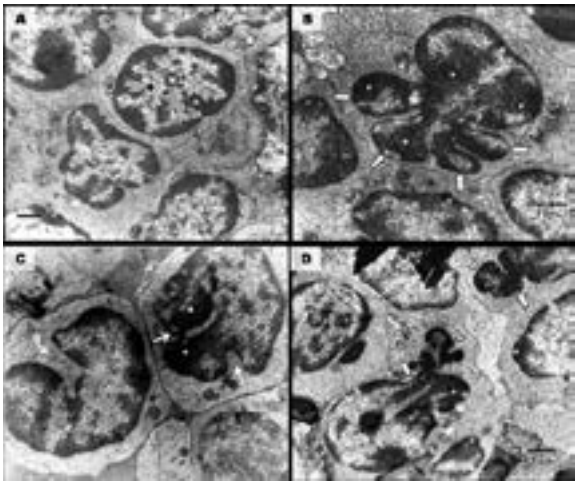

Figure 1: Ultrastructural nuclear changes in spleen lymphocytes after vanadium exposure. A. Control lymphocyte with a round nucleus, regular nuclear envelope (►), heterochromatine (✱) and eucromatine (✚) normally distributied, and one nucleoli (N) was observed in each nucleus. B. Multilobulated (⇨), C. Invaginations (→), D. Evaginations (➢) nuclei were observed after exposure. Also nuclear chromatin redistribution as well as increased and conglomerated nuclear pores were appreciated (➥).

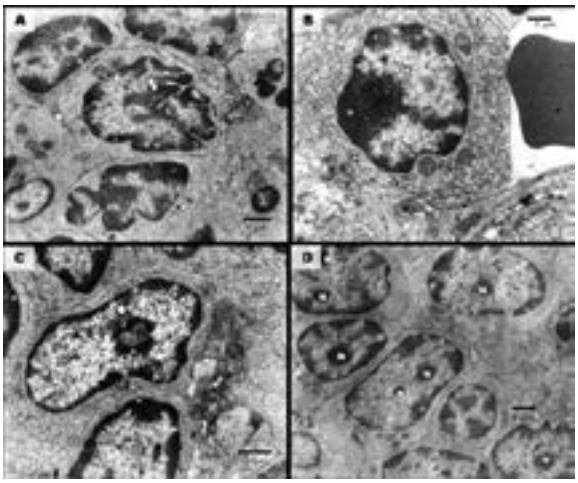

Figure 2: A. Nuclear pores increased in number and also were located in gropus. B. Nuclear edema (►), Pseudoinclusions (➥), and increased nucleoli were observed in lymphocytes from exposed animals.

Previously, our group and others reported the vanadium interaction with cytoskeletal proteins like tubulin, actin and intermediate filaments [17,18]. Cytoskeleton and nucleoskeleton are related, and remodeling of cytoskeleton intervenes in nuclear structure [19]. This relation was evidenced after the treatment of cells with taxol and vinka derived compounds (vincristine and vinblastine). Multilobulated nucleus and micronuclei were observed after the treatment; changes that resembled those observed in our study, findings that made us suggest the concurrence of cytoskeleton in the observed changes.

Other possible explanation for the nuclear changes is the interaction of vanadium with nuclear membrane proteins like MAN1, emerin, LBR, proteins in which components of the nuclear structure are affixed [20]. Reports of mutations in these proteins result in changes similar to our findings [21,22].

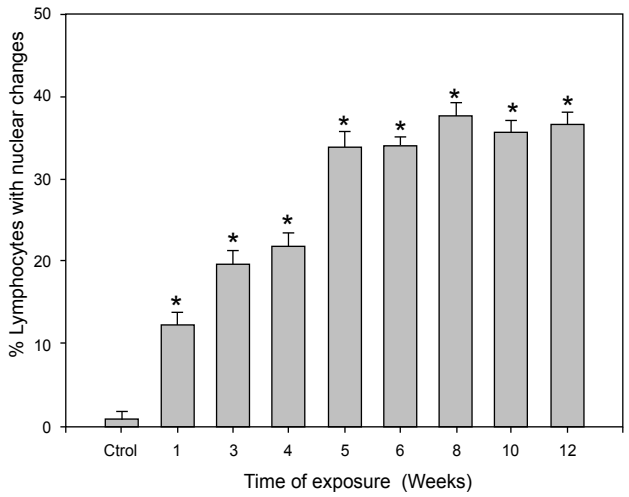

Figure 3: Frequency of spleen lymphocytes nuclear changes after vanadium exposure. A significant increase in percentage of lymphocytes nuclear changes was observed compared with controls. Changes correlated with time of exposure *=*P<0.001* ANOVA (TUKEY).

On the other hand, nucleoskeleton is a prominent structure in supporting nuclear function and integrity [23]. Several proteins build this structure, and nuclear lamins play a fundamental role. Liu et al. [24] reported in *C. elegans* that when iRNA for Ce-lamin was introduced 95% of the cells showed nuclear distortion, in addition to modifications in chromosomal distributions after mitosis, redistribution of nuclear pores, as well as areas with reorganization of DNA distribution. Nuclear lamins are members of the intermediate filaments family, and mutations in these proteins are known as laminopathies [12,25]. Nuclear aberrations in subjects with these alterations are a prominent feature; in addition, nucleolar disturbances as well as interferences in nuclear cycle proteins have been reported, but the question that remains in these syndromes is why in this patients with severe chromatin regulation changes, an increase in neoplastic changes is not reported.

It is possible for Vanadium to enter into the nuclei because its structural analogy with phosphorus and by this way replace it and modify the function and structure of enzymes, receptors, membrane channels, including DNA.

The interest in study vanadium toxic effects is because its concentrations are increasing in the atmosphere of large cities, and adding our findings to the reports by others [13] we might support the high potential that vanadium has as a neoplastic element.

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