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Research article

Alzheimer-like cell death after vanadium pentoxide inhalation

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ABSTRACT

Vanadium (V) toxicity depends on its oxidation state; it seems that vanadium pentoxide (V_2O_5) is the most toxic to the living cells. It has been reported that oral administration induces changes in motor activity and learning; in rats, I.P. administration increases lipid peroxidation levels in the cerebellum and the concentration of free radicals in the hippocampus and cerebellum. Mice that inhaled V_2O_5 presented a reduced number of tubulin+ in Leydig and Sertoli cells; it has also been reported that inhaled V_2O_5 induces loss of dendritic spines, necrosis, and hippocampus neuropil alterations; considering the direct consequence of the interaction of V with cytoskeletal components, makes us believe that V_2O_5 exposure could cause neuronal death in the hippocampus similar to that seen in Alzheimer disease. This work aimed to determine pyramidal hippocampal CA1 cytoskeletal alterations with Bielschowsky stain in rats exposed to V2O5. Male Wistar rats inhaled 0.02 M of V2O5 one h two times a week for two and six months. We found that rats, which inhaled V2O5 reached 56,57% of dead neurons after six months of inhalation; we recognize strong argyrophilic and collapsed somas and typical flame-shaped in all V-exposed rats hippocampus CA1 compared to controls. We also observe somatodendritic distortions. Axons and dendrites displayed thick dark bands replaced by noticeable thickening and nodosities and the cytoskeleton fibrillary proteins' linear traces. Our findings suggest that V2O5 inhalation induces Alzheimer-like cell death with evident cytoskeletal alterations.

1. Introduction

Exposure to metals and metalloids, such as Vanadium (V), has increased due to anthropogenic activities such as minerals processing in smelters and fossil fuel combustion. V occurs widely distributed in suspended particles due to the burning of fuel-derived products such as gasoline. Fortoul et al. [1] reported that V has increased over time in lung parenchyma from Mexico City inhabitants. There is scarce data about the consequences of this metalloid's inhalation on the population's health. Workers exposed to V have been reported to have motor and neurobehavioral deficits [2, 3]. V is a potent neurotoxin because it generates oxidative stress, lipoperoxidation, decreased antioxidant levels [4], and some antioxidant enzyme inhibition [5].

On the other hand, Alzheimer disease (AD) is described by a substantial neuronal loss in specific brain regions such as the hippocampus, the senile plaques extracellular accumulation constituted of amyloid-β peptide [6], and the occurrence of intracellular neurofibrillary tangles formed of abnormally hyperphosphorylated tau protein [7]. Tau is a microtubule-associated protein primarily expressed in axons. Its hyperphosphorylation has been indicated to provoke paired helical filaments and tangles formation [8], which correlate with AD's cognitive impairment levels [9, 10].

Earlier reports from our group demonstrated that mice, which inhaled vanadium pentoxide (V_2O_5) have a dopaminergic neuronal loss in the substantia nigra and, as a consequence, developed morphological alterations of the striatum medium-size spiny neurons [11], blood-brain barrier disruption [12], and hippocampal neurons alterations [13]. Furthermore, it seems that V_2O_5 can interact with cytoskeletal proteins, such as actin filaments, where polymerization is inhibited [14] and is also a potent inhibitor of tyrosine hosphatases [15], which in turn,

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decreased gamma-tubulin, which disturbs the formation and function of microtubules [16]. It is also well known that the actin cytoskeleton polymerization determined dendrites and spines morphology [17]. Thus, considering V interaction's direct consequence with cytoskeletal components makes us believe that V_2O_5 exposure could cause neuronal death in the hippocampus similar to that seen in AD.

2. Material and methods

The experiments were conducted in 12 male Wistar rats weighing 180–200 g. The rats were individually housed in plastic cages following controlled light conditions (12 h light/h dark regime) and fed with Purina Rat Chow and water *ad libitum*. Bodyweight was recorded daily. The experimental protocol was conducted according to the Animal Act of 1986 for Scientific Procedures and approved by the UNAM Etic Committee (NOM-062- ZOO-1999, México (approbation number: 1136)). All efforts were made to minimize the number of animals used and their suffering. V_2O_5 inhalations were carried as described by Avila-Costa et al. [11]. Six rats were placed randomly in an acrylic chamber inhaling 0.02 M V_2O_5 (Sigma, St. Louis, MO, USA) 1 h twice a week for two and six months; control rats (n = 6) inhaled deionized water for the same time.

After two or six months, rats were perfused under sodium pentobarbital anesthesia via the aorta, with a saline solution followed by the fixative containing 10% formaldehyde in 0.2M-phosphate buffer (PB). The brains were removed and placed in the fixative solution for one hour.

Bielschowsky silver impregnation method: Following routine processing in paraffin, serial coronal brain sections were cut at 8 μm thickness in a sliding microtome (Leica SM2010 R, Germany). Brain sections were deparaffinized through xylene and alcohols into tap water before being placed into 20% silver nitrate solution for 20 min at 37 °C. After washing with distilled water, slides were immersed in 20% silver nitrate solution titrated with fresh sodium hydroxide and evaporated ammonia. After 15 min, slides were washed with ammonia before being individually revealed with 100 ml of a developer (20 ml of formaldehyde, 100 ml distilled water, 20 μl concentrated nitric acid, and 0.5 g citric acid) and then added to 50 ml of titrated silver nitrate solution. Slides were then rinsed in tap water, fixed in 5% sodium thiosulfate, and dehydrated through alcohols and xylene [18]. All of the pyramidal cells in the CA1 region were counted under a light Optiphot 2 microscope (Nikon, Japan) at 40× magnification.

One-way ANOVA was used to analyze the number of damaged cells. Group differences were considered statistically significant at $P < 0.05$. When appropriate, *posthoc* comparisons were made with the Tukey test. All analyses were conducted with GraphPad Prism 8 Software.

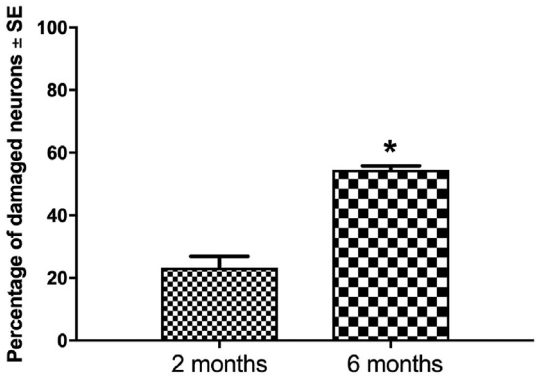

Figure 1. Mean percentage of the total number of hippocampus CA1 damaged neurons after two or six months of V_2O_5 inhalation. *$P < 0.05$ vs. two months group.

3. Results

After two and six months of V_2O_5 inhalation, neither clinical changes nor weight differences were identified in the exposed animals compared to controls.

With the Bielschowsky silver impregnation method, we observed that rats that inhaled V_2O_5 after two months have significant pyramidal hippocampal cell death (25%), and after six months, the neuronal death reached 56.57%, being statistically different vs. two months and control groups (Figure 1); intense argyrophilic and collapsed somas in all V-exposed rats hippocampus CA1 is noticeable compared to control rats (Figures 2 and 3). With the Bielschowsky method, we also recognize strong argyrophilic and collapsed somas and typical flame-shaped in all V-exposed rats hippocampus CA1 compared to controls (Figures 1 and 2). Also, somatodendritic distortions were identified. Axons and dendrites displayed thick dark bands replaced by noticeable thickening and nodosities and the linear traces made by the cytoskeleton fibrillary proteins. The neurofibrils were found fused, disordered, thickened, and crowded together into broadband, and the neurites were deeply stained; we observed curly fibers also. Some neurites displayed neurofibrillary-type tangles (Figure 4).

Our observations demonstrate a prominent impairment of the cytoskeleton of many nerve cells in the V-exposed rats, similarly to what occurs in some neurodegenerative processes.

4. Discussion

Vanadium's neurotoxic effects have been mainly attributed to its ability to induce the generation of reactive oxygen species (ROS) [4] and neuron inflammation [Jaiswal and Kale, 2020]. It is noteworthy that the neurotoxicity caused by occupational V exposure commonly occurs with co-exposure to other metals, especially manganese (Mn) [19]. It has been reported that in brain cell cultures, V compounds induce apoptosis, DNA cleavage and promote iron-mediated oxidative stress [4]. In general, V neurotoxic effects are summarized in Figure 5.

When V enters the body through inhalation [20], it can enter as pentavalent (Vanadate) or tetravalent (vanadyl) forms; in the blood, it is transported by transferrin and albumin (**1**). These two forms enter the cells through anionic channels, once inside the vanadate reacts with antioxidant enzymes such as SOD (**2**), generating ROS, H_2O_2 through Fenton reaction, triggers the mitochondrial Cytochrome C pathway to activate the caspase 9 and 3 pathways and the apoptotic pathway (**3**) [21], CAT and GSH enzymes react with vanadate to generate free radicals

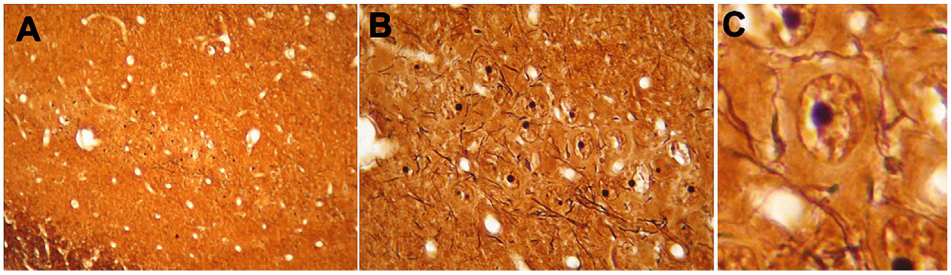

Figure 2. Representative microphotographs of Bielschowsky staining from Hippocampus CA1 control group. A 10x, B 40x and C 100x.

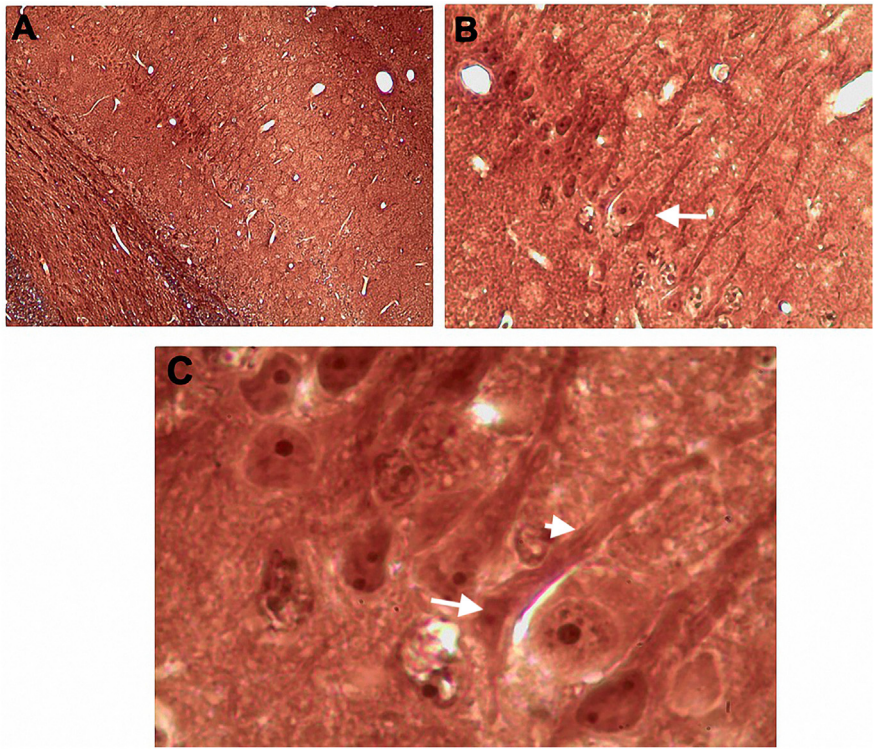

Figure 3. Representative microphotographs of Bielschowsky staining from Hippocampus CA1 experimental group after two months of V_2O_5 inhalation. Somato-dendritic deformation is observed (arrows). The axons have thicker and darker bands (arrowhead); A (10x), B (40x), and C (100x).

(OH^+ OH^-) (**4**) [4], inducing oxidative stress causing alteration in lipids, proteins, and nucleic acids. NADPH-oxidase can reduce vanadate to the vanadyl form (**5**), which in turn, oxidized by H_2O_2, forms pervanadate that will irreversibly inhibit protein tyrosine phosphatases (PTP) [22] (**6**), it will activate intracellular signaling pathways, increasing the phosphorylation of protein tyrosine kinase (PTK) (**7**) [23], triggering the inflammation mechanisms through the formation of phospholipase-A2 (PLA-A2) and COX-2, inducing the activation of microgliosis and astrogliosis (**8**) [24], likewise the lipoperoxidation of the cell membrane-inducing cell death, demyelination and damage to lipids, proteins, and nucleic acids. Unreduced vanadate inactivates protein tyrosine phosphatases (PTPs) (9) [25], which leads to the activation of intracellular signaling pathways (see Figure 5).

It is important to note that V concentrations in ambient air vary considerably; in rural areas, the levels are below 0.001μg/m^3, however, in areas where there is a high degree of fossil fuels burning, as in large

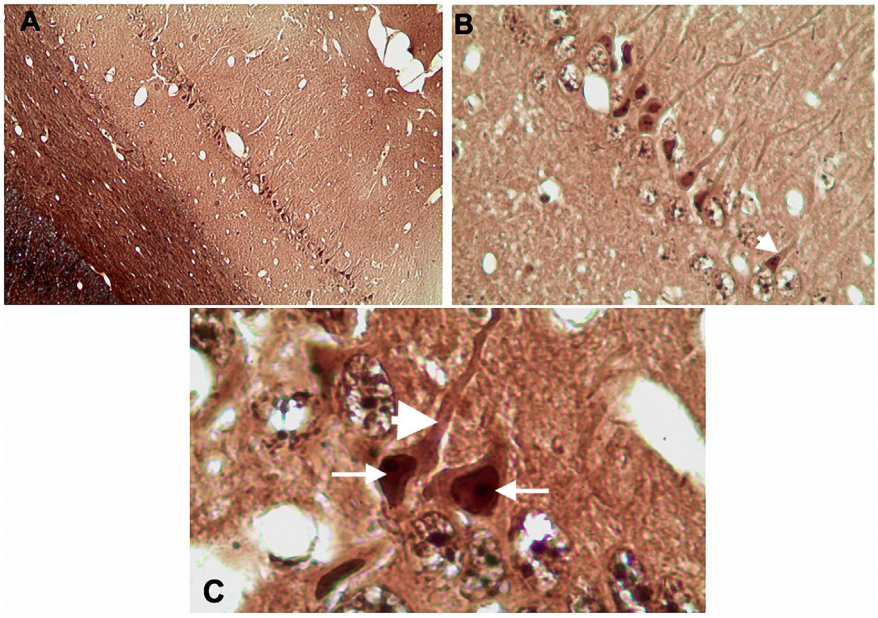

Figure 4. Representative microphotographs of Bielschowsky staining from Hippocampus CA1 experimental group after six months of V_2O_5 inhalation. It can be observed strong argyrophilic (arrows) typical flame-shaped and intensely stained neurites, forming similar structures to neurofibrillary tangles (arrowheads); A (10x), B (40x), and C (100x).

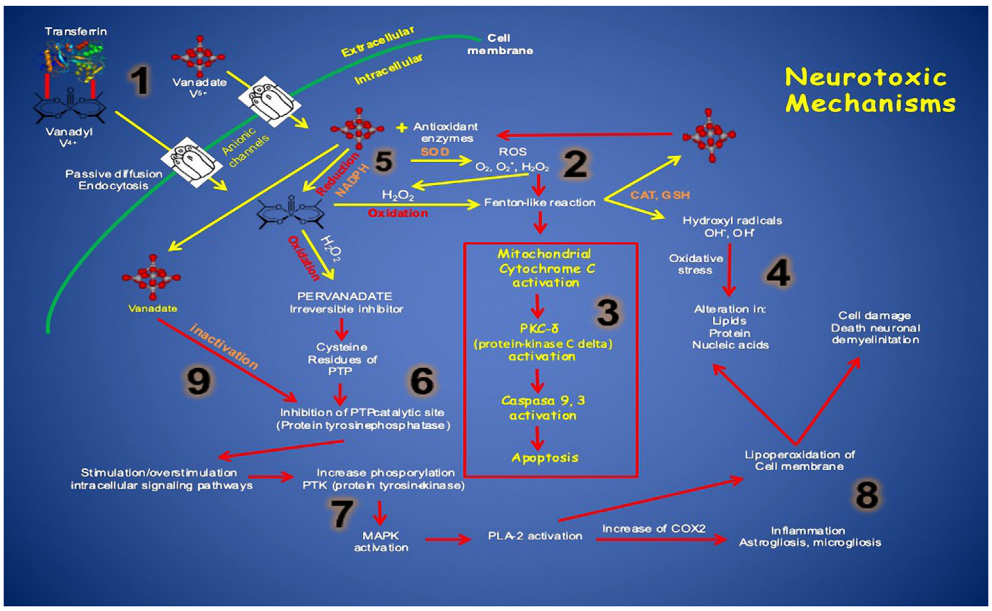

Figure 5. Vanadium neurotoxic mechanisms.

cities, the average annual concentration goes from 0.02 μg/m^3 to 0.3 μg/m^3; It has been determined that in the vicinity of industrial zones its level can reach 1 μg/m^3 [1]. In our laboratory, the concentration of vanadium in the inhalation chamber was determined, when 0.02m of V_2O_5 was administered, as in the current experiment, finding that the average concentration was 1436 μg/m^3 [16], above the highest concentration detected in ambient air (1 μg/m^3).

Moreover, as previously reported, V alters different cytoskeletal proteins such as γ-tubulin [16] and can produce actin changes [14]. Several earlier studies have demonstrated that some V compounds are associated with actin. This cytoskeletal protein has high-affinity binding sites for this metal. Oxovanadium (IV), for instance, interacts with F-actin and G-actin with 1:1 and 4:1 stoichiometries, respectively, and it has been suggested that the interaction of VO2þ with G-actin may happen near the actin adenosine triphosphate binding position [26, 27, 28]. Also, decavanadate can alter actin's structure by oxidizing its cysteines in its polymerized form [29].

Notably, earlier reports showed that V compounds might induce Tau hyperphosphorylation [30, 31] and oxidative stress [32], resulting in AD-like damage.

Moreover, the significant hippocampal cell alterations could result from the availability of G-actin for V, and its association with the metal, because neurons have an extremely dynamic cytoskeleton, requiring continuous actin filaments polymerization [33].

In conclusion, the present study demonstrates that V_2O_5 inhalation significantly induced "neurofibrillary"-type tangles associated with the main pathological AD changes [34] in exposed rats hippocampus. Thus, more studies are needed to determine the relationship between V_2O_5 inhalation and Tau hyperphosphorylation, not only in the hippocampus but also in the entorhinal cortex, amygdala, and neocortex, structures involved in AD [35], and whether there are alterations in spatial memory.

Declarations

Author contribution statement

Enrique Montiel-Flores, Claudia Dorado-Martínez: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data.

Oscar A. Mejía-García: Performed the experiments.

Jose Luis Ordoñez-Librado, Ana Luisa Gutierrez-Valdez, Leonardo Reynoso-Erazo, Rocio Tron-Alvarez: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Jesús Espinosa-Villanueva: Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Vianey Rodríguez-Lara: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Maria Rosa Avila-Costa: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

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Data availability statement

Data will be made available on request.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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