Chapter

Alzheimer-Like Cell Alterations after Vanadium Pentoxide Inhalation

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Abstract

Vanadium (V), a widely distributed transition metal, has been considered toxic, which depends on the valence of the compound. V pentoxide (V_2O_5) is considered the most harmful. Its long-term exposure produces neurotoxicity. Mice exposed to inhaled V_2O_5 displayed less tubulin$^+$ in testicular cells and dendritic spines loss, cell death, and CA1 neuropil modifications, considered as the result of V interaction with the cytoskeleton, which made us suppose that V_2O_5 inhalation could initiate CA1 cell alterations comparable to what happen in the brains of Alzheimer disease (AD) patients. This study intends to demonstrate pyramidal CA1 cytoskeletal changes in rats which inhaled V_2O_5. Twenty rats were exposed to V_2O_5 0.02 M one hour, three times a week for several months. Our findings showed that V_2O_5-exposed rats had cell death that reached 56,57% after six months; we also observed collapsed strong argyrophilic nuclei and characteristic flame-shaped somas in all V_2O_5-exposed animals hippocampus CA1 compared to controls. We also found somatodendritic deformations. Neurite's cytoskeleton exhibited visible thickening and nodosities and prominent dendritic spine loss. Our results demonstrate that V_2O_5 induces AD-like cell death with evident cytoskeletal and synaptic alterations.

Keywords: Vanadium pentoxide, Cell death, Bielschowsky silver stain, inhalation, dendritic spines, hippocampus

1. Introduction

Vanadium (V) is a transition metal abundant in nature; its atomic number is 23. Andres Manuel Del Rio was the first who reported it in 1801. But it was actually discovered in 1830 by a Swedish chemist named Nils Sefstrom [1]. V is a bright silver-white, soft and malleable metal and the 22nd most abundant element in the earth's crust, and it has become a matter of concern among nutritionists since various marine species contain this metal as an trace element [2]. Environmental air V acts as the primary source for the general population [3].

Although V is extensively dispersed in air, its role as human nutrient is not yet confirmed. Humans are exposed to V generally through the polluted atmosphere from combustion products of vanadium-bearing fuel oils, fumes, and dust. Food contains insignificant V concentrations, frequently below 1 ng/g. V enters the organism by inhalation, skin, and gastrointestinal tract and accumulates mainly in the kidney, liver, bones, spleen, lungs and brain, accumulate fewer V concentrations [3–5].

Neurotoxic effects of V are not well recognized yet. Still, it is known that acute exposure in animals by ingestion or inhalation leads to nervous system alterations, paralysis of legs, respiratory failure, convulsions, bloody diarrhea, and death [6]. V disrupts the blood–brain barrier [7] and alters some neurotransmitters concentrations such as serotonin, norepinephrine, and dopamine, and an inhibitory effect on the uptake and release of norepinephrine were observed in the rat brain during V poisoning [8–10].

The V oxidation states of biological importance are vanadate (V^{5+}) and vanadyl (V^{4+}) and are considered harmful to mammals depending on their levels. Workers occupationally exposed to vanadium pentoxide (V_2O_5) had presented cardiovascular alterations and a variety of symptoms involving the central nervous system (CNS), gastrointestinal and respiratory systems [11]. Moreover, it has been suggested that raised tissue levels of V may be of etiological importance in manic-depressive syndrome since V reduces serotonin concentration. Blood V levels in depressed patients were greater than non-V-exposed controls [11]. Besides, reduced cognitive abilities in humans chronically exposed to this metal were found [12].

2. Vanadium sources

Metallic V is not found in nature. The most common in mining is carnotite and vanadinite. V is also found in phosphate rock, iron ores, and some crude oils in organic complexes and in small percentages of meteorites [3]. The presence of V is related to other minerals; among them is iron, aluminum, uranium, and titanium, and is frequently used as alloy steel, in combination with nickel, boron, or manganese. Extraction of V from coal or fossil fuels, such as Vanadium-rich coal tars and oil, explains the high V concentrations registered in the atmosphere [11].

V is generally employed in metallurgy in alloy with steel. And, as nonferrous metal V is considered fundamental for aircraft's manufacture, atomic and space industries. In the chemical industry, V_2O_5 and metavanadates are remarkably important for plastics and sulfuric acid production. Emissions of V may be high near producing steel alloys industries. V is also released into the air: during the resmelting of scrap steel and the transformation of titaniferous and vanadic magnetite iron ores into steel; from the roasting of V slags; from V_2O_5 smelting furnaces; and from electric furnaces in which ferrovanadium is smelted [11, 13].

2.1 Vanadium in the environment

As a profuse element in the earth's crust, the V average varies from 159 g/t to 0.14 mg kg. The standard concentration of 135 mg/kg in soil positions V in 5th place, among all transitional metals [11, 13]. V recycling includes its release from anthropogenic and natural bases to the water, soil, and air [13–15]. Frequently, the places such as fuel plants and refineries showed the highest level of V [16, 17].

V geochemical characteristics depend on the oxidation state and pH. The moderately immobile V (III) prevails. Typically, V compounds with high oxidation states are more soluble [14]. The average concentration of V in different soils

fluctuates from 10 to 220 mg kg dry mass depending on the soil types and chemical characteristics [18, 19]. The soils directly under humans' use include a much higher V concentration [17, 18]. On the other hand, what most pollutes the soil and water is the mining V-derived [20]. Vanadium is the most profuse transition metal in the aqua sphere, with an average content similar to zinc [21]. Persian Gulf sediments have very high V concentrations [22].

It seems that over the last decades, V levels in the biosphere have been significantly growing, a fact that will be of concern in the future [23]. The primary sources are mining, fossil fuel combustion, atmospheric wet and dry accumulation, etc. [24]. V remains in the water, soil, and air for long periods and may react with other elements [2, 21]. Recently, it has been shown that atmosphere V levels are increasing every day, mainly due to fossil fuel burning [11, 14, 18]. For that reason, more than 60 thousand tons of V may be released into the big cities air [14, 25].

Apparently, V concentrations in ambient air fluctuate significantly; in rural areas, V levels are under 0.001 μg /m^3, however, in areas where there is a high degree of fossil fuel burning, as in large cities, the average annual concentration goes from 0.02 μg/m^3 to 0.3 μg/m^3. It has been determined that near industrial zones, its level can reach 1 μg/m^3 [26]. Fortoul et al. [26] reported that V has increased over time in lung parenchyma from Mexico City inhabitants since it has been demonstrated that Mexican petroleum has high V concentrations.

V concentration in plants and food is very low, from less than 0.001 to 0.005 mg [14]. Some foods, including oysters, parsley, and spinach, had a relatively higher amount of V than all other foods [27].

V occupational exposure. V levels near metallurgical industries usually average about 1 mg V/m^3, whereas ambient air near industries, which produce V metal or compounds, contain a few mg V/m^3 [11]. Very high levels of V result from boiler-cleaning procedures due to the high concentration (approximately 10–25%) of V oxides in the dust. During these procedures, 50–100 mg V/m^3 are frequent, with concentrations ranging from 500 mg V/m^3 [3].

The most critical V compounds are ferrovanadium, V_2O_5, vanadium trioxide, V carbide, and salts, such as ammonium and sodium vanadate. The salts and oxides are used in powder form. It has been reported that the metallurgical industry includes the production of vapor containing V_2O_5, which condenses to form breathable aerosols. Also, residual fuels combustion with high V content have V_2O_5 aerosols [11].

2.2 Vanadium absorption, distribution and excretion

It appears that only 10% of ingested V is absorbed from the gastrointestinal tract [28]. This report suggests that most of the ingested V is transformed into the cationic vanadyl form in the stomach before being absorbed in the duodenum through an unknown mechanism [29]. In its anionic vanadate form, V is absorbed in much higher quantities (about five times more than vanadyl form) through an anionic transport system [29]. Multivalent existence of V in nature and living systems put forth the chemical complexity of this element. This multifaceted chemical character of V, in turn, echoes in its biological and biochemical properties, especially in metabolism and absorption. Again vanadate, after reaching the bloodstream, is converted into vanadyl ion, although the vanadate form also exists. Thus, vanadate (by transferrin) and vanadyl (by albumin and transferrin) are rapidly transported by blood proteins to various tissues [30]. Blood parameters showed little or no reflection of toxicity after a long-term supplementation of V compounds [31], which might be due to the transport of V from blood to the tissues. Upon supplementation, V is incorporated in various organs and tissues, including the liver,

kidney, brain, heart, muscles, and bone. The kidney, spleen, bone, and liver tissues of rats have been shown to accumulate distinctly high amounts of V in chronically treated animals through oral administration [32].

The effects of V persist even after it has been withdrawn for several days [33]. Unabsorbed V is excreted in feces. When V was administrated through the parenteral route, 10% of the V was found in the feces of humans and rats [3]. V is excreted through bile and urine [34]. It is, thus, the bile route through which a significant amount of V may be eliminated through feces. Moreover, it may be suggested that V content in feces does not reflect V absorbed or unabsorbed (1).

The toxicity of V depends on various factors, including the administration route and the V compound toxicity. In general, the toxicity of V is low, and its toxicity is least following ingestion and greatest following parenteral administration. Inhalation is a route of exposure that produces intermediate toxicity [3, 11]. The toxicity of V increases with higher valences, and the pentavalent compounds (as V pentoxide) are usually the most toxic [3].

2.3 Vanadium effects in the nervous system

V crosses the blood–brain barrier [7], and its compounds can induce neurologic alterations through different routes of administration [3, 11]. It has been reported that V-exposed lactating rat pups developed neurological deficits [35]; other authors described neurological alterations and increased brain V concentration after sodium metavanadate intraperitoneal administration [36–38]. Also, our group [7, 8] reported neuroinflammation in the brain of mice that inhale V_2O_5. We found a seven-fold peak increase in V brain concentration after one week of inhalation and remained constant (0.10–0.12 mg/g dry weight tissue) during eight weeks of V_2O_5 inhalation. The inhalation route seems to induce neurotoxicity [6], which is epidemiologically relevant since this is the main route to the brain during occupational and environmental exposure.

One of the first studies on the V neurological effects was made by Done [39], who found that humans exposed to V displayed tremor and depression. Other researchers demonstrated that occupationally exposed people present alterations in cognitive ability tasks [40]. Despite the route, duration, and compound, V exposure has affected nerve cells and glia. In a study of chronic intraperitoneal exposure at 3 mg/kg in mice, Folarin et al. [36] reported that the brain accumulates large amounts of V, mainly in the brain stem, cerebellum, and olfactory bulb. This study described disruption of the layering pattern in the prefrontal cortex with nuclear pyknosis, loss of pyramidal neurons and reduced apical dendrites in the hippocampal CA1, and loss of cerebellar Purkinje cells. These morphological alterations were accompanied by astrogliosis and microgliosis.

Demyelination has also been reported after drinking milk from mothers exposed to sodium metavanadate [41]. Our group also described that in male CD-1 mice exposed by inhalation to 0.02 M V_2O_5 2 h twice a week for four weeks, Golgi staining revealed a severe loss in dendritic spines in the striatum compared to the controls, showing that the inhalation of V_2O_5 causes severe neuronal damage in this nucleus [8]. We observed fewer dendritic spines in the olfactory bulb granule cells after three months of exposure using the same inhalation protocol, and electron microcopy alterations consisted in swelled mitochondria and endoplasmic reticulum, and neuronal death that can be correlated with the olfactory dysfunction [42]. In the hippocampus, we found a decrease in dendritic spines and necrosis of the pyramidal CA1 neurons, modifications that could be associated with spatial memory impairment [43].

2.4 Mechanisms of vanadium neurotoxicity

It has been reported that V induces reactive oxygen species (ROS) production, which several authors have proposed as a reasonable basis for its neurotoxicity [6, 44, 45]. V, as other catalytic transition metals, participate in the Fenton reaction [46]. V in body fluids exists mainly in the 5^+ oxidation state as V pentoxide (V_2O_5) [47]. V enters the cell as vanadate via anion channels while as vanadyl ions by passive diffusion and endocytosis bound to transferrin [48]. When entering the cell, vanadate is reduced by intracellular antioxidants to vanadyl, with subsequent production of ROS [49]. H_2O_2 then oxidizes vanadyl into vanadate in a Fenton-like reaction with the consequent hydroxyl radical production [50]. With higher V levels, these reactions result in oxidative stress and toxic effects on lipids, proteins, and nucleic acids. With its high lipid content, the brain is vulnerable to oxidant-induced lipid peroxidation [51], and as such, V neurotoxicity is related to myelin deficits [45]. Moreover, as we mentioned above, earlier results from our group revealed substantia nigra tyrosine hydroxylase cell loss, and therefore, dendritic spine loss in the striatum medium-size spiny neurons [8], blood–brain barrier disruption [7], and hippocampal cells alterations [43].

Besides oxidative stress, it has been demonstrated that the cytoskeleton is an important target of V toxicity because of its ability to compete with phosphatases; due to this, V inhibits actin polymerization through the tyrosine phosphatases inhibition [52, 53], which, in consequence, by decreasing gamma-tubulin disturbs microtubules function and formation [54]. It is also well known that actin polymerization establishes the morphology of dendrites and dendritic spines [55]. These facts make us consider the possibility that V_2O_5 inhalation might induce hippocampus cell death similar to that seen in Alzheimer disease (AD).

2.5 Alzheimer disease

Today, aging human populations worldwide face an epidemic of AD, with an increasing number of cases to nearly 106 million by 2050 [56]. Several factors have been described to participate in the AD etiology including, aging, genetics [57], head injury [58], and exposure to certain chemicals and compounds [59].

AD is a neurodegenerative disease that represents the most common cause of dementia. Symptoms associated with dementia vary from difficulties with orientation, language, and problem-solving to memory alterations and other cognitive skill deficits that affect a person's ability to perform daily life activities [60]. The most noticeable symptoms at the beginning of the disease are disorientation and episodic and spatial memory loss [61]. The medial temporal lobe region, consisting of the hippocampal formation and related cortices, are essential for the adequate functioning of spatial and declarative memory systems [62, 63] and are the first areas affected in the progression of the disease [64].

Synaptic failure has been suggested as the leading cause of AD pathology [65]. The principal neuropathological hallmarks of the disease are the neurofibrillary tangles (NFTs) associated with abnormal phosphorylated tau protein and the accumulation of aberrant amyloid-β, features also found in the brains of old patients without cognitive impairments or AD [66]. Nonetheless, directly or indirectly, these proteins induce synapsis alterations by changing dendritic spines morphology or causing their loss and neuronal degeneration [67, 68].

The development of intraneuronal lesions at selectively vulnerable brain structures is central to the pathological process in AD [69–71]. The lesions consist mainly of hyperphosphorylated tau protein. They include tangle material, NFTs in cell

bodies, neuropil threads (NTs) in neuronal processes, and material in dystrophic nerve cell processes of neuritic plaques (NPs) [72–74].

2.6 Alzheimer's disease experimental models

Experimental models are crucial in understanding AD pathogenesis for implementing novel therapeutics. So far, AD experimental models consist almost exclusively of transgenic mammals that express the human genes that result in the formation of amyloid plaques (by expression of human APP alone or in combination with human PSEN1) and NFTs (by the expression of human MAPT) [75–78]. Other experimental models have used invertebrates such as C. *elegans and Drosophila melanogaster* and vertebrates such as zebrafish; nevertheless, these models are very different from human physiology and less extensively used [79]. Nevertheless, some issues have been raised about this model's validity, mainly because the efficacy in clinical trials has been very low [80, 81]. Facts that make us wonder if the animals in the experimental models actually have AD, considering only the specific pathological features. Most animal models develop only the amyloid accumulation that defines AD. This often gives rise to specific memory-associated cognitive alterations. However, these models normally preset the absence of the main AD pathological features, including cell death and, most importantly, NFTs development [79]. The lack of NFTs could partly explain the failure between pre-clinical and clinical trials [80].

Therefore, in this chapter, we intend to demonstrate that the inhalation of V_2O_5 produces cellular alterations like those observed in AD, with synaptic alterations (shown by the loss of dendritic spines) and by the presence of NFTs, due to V directly interacts with the cytoskeletal components, and is a potent inhibitor of tyrosine phosphatases.

3. Experimental procedures

The experiments were accomplished in 24 male Wistar rats weighing 180–200 g at the beginning of the study. The rats were individually placed in plastic cages with controlled light conditions (12 h light/12 h dark) and fed with Purina Rat Chow and water *ad libitum*. Body weight was recorded daily. The experimental protocol was carried out following the Animal Act of 1986 for Scientific Procedures and the Rules for Research in Health Matters (Mexico). We made efforts to minimize the number of animals used and their suffering.

3.1 Vanadium pentoxide inhalation

V_2O_5 inhalations were performed as described by our group [8]. As part of our experiment with V, a pilot study was implemented with 0.005 and 0.01 M V_2O_5, and we found no changes using light microscopy in lung tissue; therefore, a higher dose was utilized, 0.02 M, realizing that V half-life about 48 h [11] we designed a three times a week exposure protocol.

Twelve rats were placed in an acrylic chamber inhaling 0.02 M V_2O_5 (Sigma, St. Louis, MO, USA) (Sigma Aldrich, Co. Mexico) 1 h three times a week for two and six months. Twelve control rats inhaled only the vehicle—deionized water—for the same time. Inhalations were performed in closed acrylic boxes (40 cm wide x 70 cm long and 25 cm high) attached to an ultra-nebulizer (Shinmed, Taiwan), with 10 l/min continuous flux. The ultra-nebulizer is designed to produce droplets in a 0.5–5 μm range. A trap for the vapor was located on the opposite side with a

solution of sodium bicarbonate to precipitate the remaining metal. During the inhalation, animals were constantly monitored for respiration rate, depth, and regularity. The exposure system was monitored for temperature, oxygen level, and V concentration.

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

3.2 Bielschowsky silver impregnation

After the routine paraffin processing, serial coronal brain sections were cut at 8 μm thickness in a sliding microtome (Leica SM2010 R, Germany). Brain sections were deparaffinized in xylene and alcohol before being disposed into 20% silver nitrate solution for 20 min at 37°C. After washing with distilled water, slides were submerged 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 [82]. The hippocampus CA1 pyramidal cells were evaluated under a light Optiphot 2 microscope (Nikon, Japan).

3.3 Golgi stain

Brain tissue from the hippocampus CA1 was cut into 90 mm- thick sections and processed for the rapid Golgi method [83]. The histological analysis consisted in counting the number of dendritic spines in a 10 mm-long area from five secondary dendrites from 20 CA1 pyramidal neurons from each rat [8, 84].

Means from each group were compared for statistical differences by one-way ANOVA test ($p < 0.05$) followed by *posthoc* comparisons with Tukey test. The statistical analyses were conducted with GraphPad Prism 9 for Mac Software.

4. Results

The animals that inhaled V_2O_5 did not show changes in their weight or clinical alterations compared to the control group.

4.1 Dendritic spines

Brain sections were treated with the Golgi stain to determine if V_2O_5 inhalation induces synaptic alterations in the hippocampus CA1. The synaptic damage resulted in significant CA1 pyramidal neurons dendritic spine loss of exposed rats compared to controls (**Figures 1** and **2B**, **C**). As it is shown in **Figure 1**, spine loss was more evident with longer inhalation time.

4.2 Hippocampus CA1 neuronal alterations

With the Bielschowsky method, we found that rats exposed to V_2O_5 after two months have substantial CA1 pyramidal cell death (25%) (**Figures 3** and **5**), and after six months, the cell death reached 56.57%, being statistically different vs.

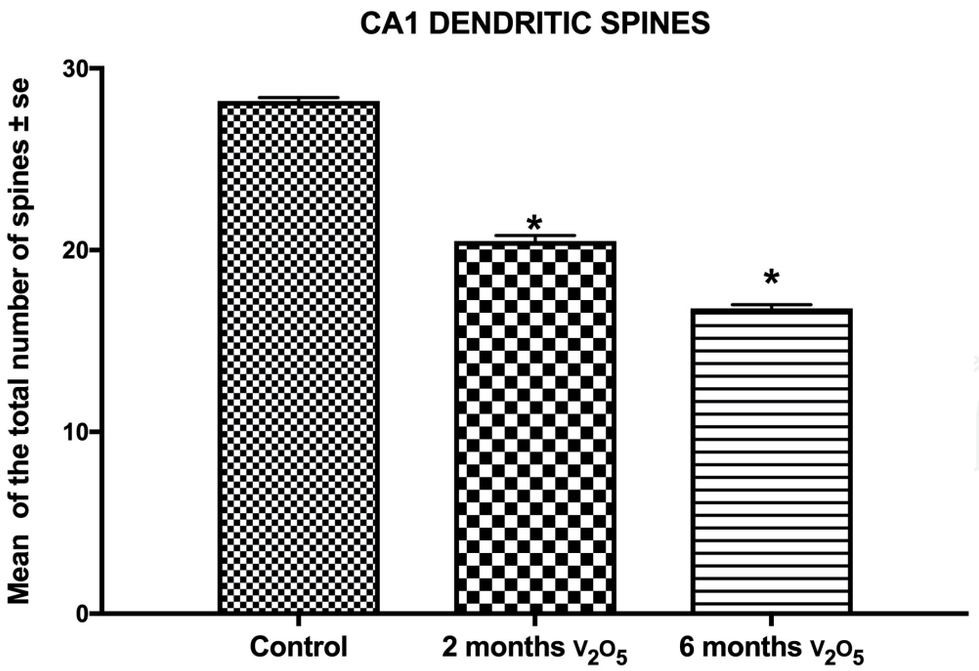

Figure 1.
*The number of pyramidal CA1 neurons dendritic spines, contrasting control and exposed rats after two and six months of V_2O_5 inhalation. One way ANOVA, *p < 0.05 vs. control group.*

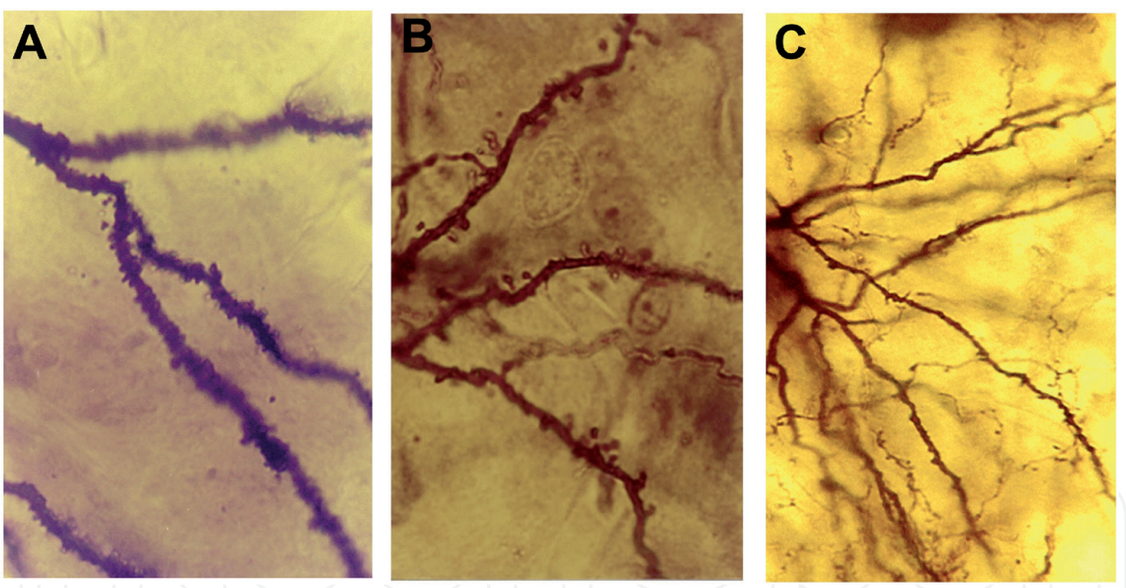

Figure 2.
Dendritic spine density. Representative Golgi-stained pyramidal CA1 neurons of the control group (A), two months (B), and six months of V_2O_5 inhalation (C). Both exposure times provoked a significant decrease in the total number of spines, mainly after six months. (magnification 40X).

two months and control groups (**Figures 3** and **4**); we observed that in all V_2O_5-exposed rats the pyramidal hippocampus CA1 cells displayed strong argyrophilic and collapsed somas compared to control rats, the somas also revealed the typical flame-shaped (**Figures 4–6**). Also, somatodendritic deformations were identified. Axons and dendrites exhibited thick dark bands resembling thickening nodosities and fibrillary cytoskeleton proteins linear traces. The neurofibrils were fused, disordered, thickened, and crowded together into broadband, and the neurites were deeply stained; we also noticed curly fibers. Some neurites displayed neurofibrillary-type tangles (**Figure 6**).

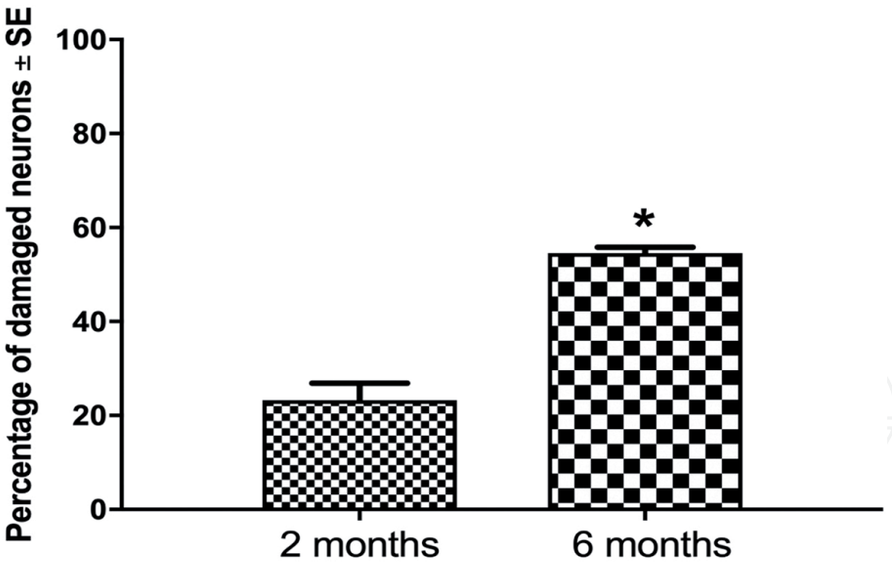

Figure 3.
*Damaged pyramidal hippocampus CA1 neurons percentage after two or six months of V_2O_5 inhalation. *P < 0.05 vs. two months group.*

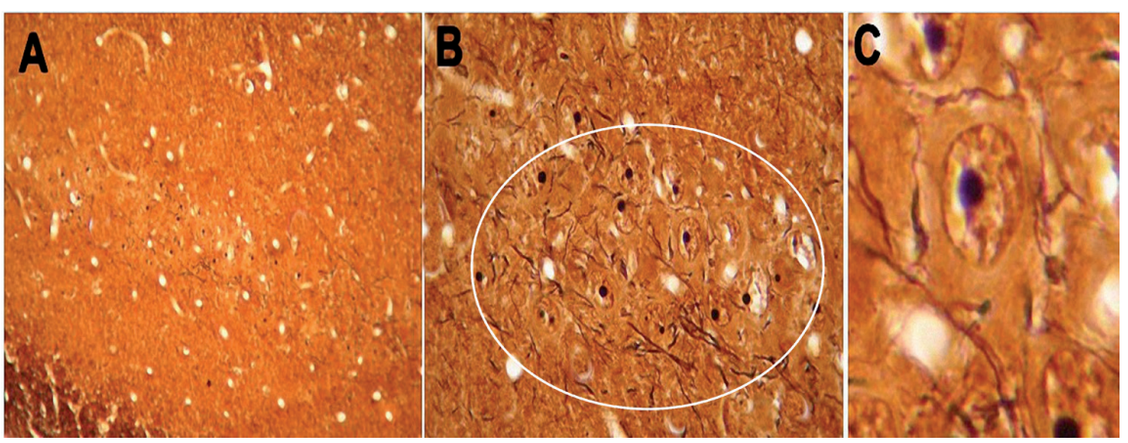

Figure 4.
Representative photomicrographs of Hippocampus CA1 control group stained with the Bielschowsky method. As can be seen in B (white oval), the pyramidal neurons of the hippocampus CA1 are healthy, in terms of size and shape. Figure C depicts the detail of B white oval. A 10X, B 40X and C 100X.

5. Discussion

Our results show significant alterations in the cytoskeleton and synaptic activity, demonstrated by the loss of dendritic spines and Alzheimer-like fibrillary tangles.

It is essential to stand out that V concentrations in the environment vary substantially; in rural areas, V concentrations are below 0.001 μg/m^3, in big cities, where there are high levels of fossil fuel burning, the average V concentration range from 0.02 μg/m^3 to 0.3 μg/m^3. It has been shown that near industrial zones, its concentrations can reach 1 μg/m^3. In this experiment, V concentrations in the inhalation chamber was 1436 μg/m^3 [54], exceeding the highest concentration reported in ambient air (1 μg/m^3). In this regard, we know that the concentrations used here are higher than those subjects with occupational exposure, but animal models permit amplifying the impact that V has on the nervous system.

Our results demonstrated that V_2O_5 inhalation generates a significant loss of pyramidal CA1 neurons dendritic spines and notorious cytoskeleton distortions resulting in the alteration of the synaptic transmission and, therefore, possibly in

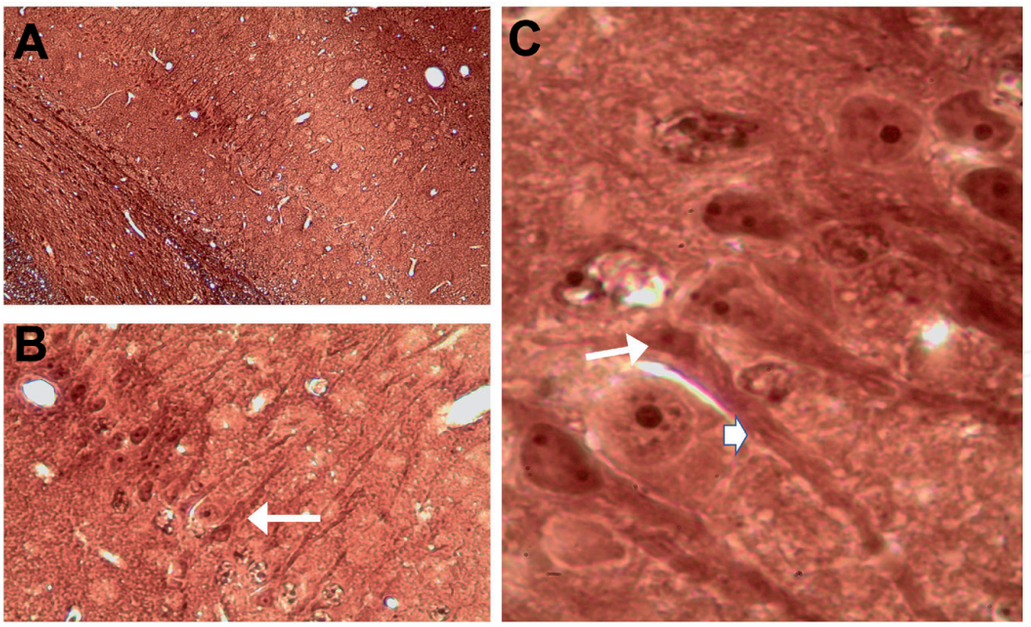

Figure 5.
Representative photomicrographs of Hippocampus CA1 Bielschowsky staining from the experimental group after two months of V_2O_5 inhalation. Neuronal soma deformation is observed (arrows). The axons displayed thicker and darker bands (arrowhead); A (10x), B (40x), and C (100x)

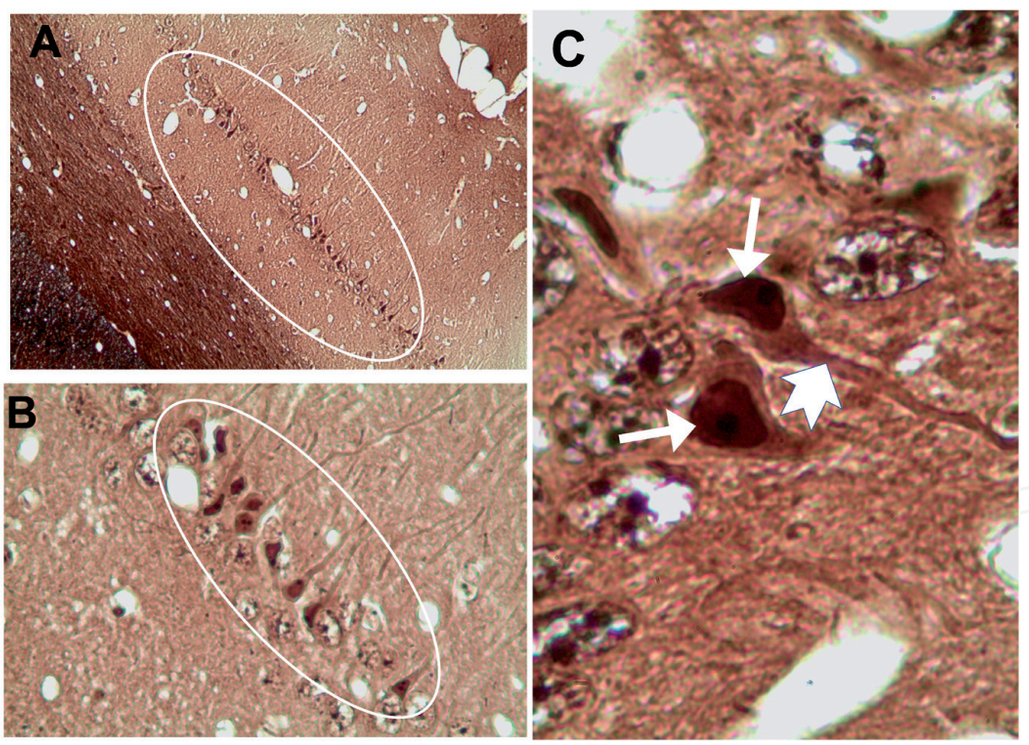

Figure 6.
Hippocampus CA1 representative photomicrographs of Bielschowsky staining from the experimental group after six months of V_2O_5 inhalation. It can be observed strong argyrophilic nuclei (white oval in a and B; arrows in C) typical flame-shaped and intensely stained neurites (white oval in a, B and C), forming similar structures to neurofibrillary tangles (arrowhead); A (10x), B (40x), and C (100x).

memory disturbances. It is well known that many neurological conditions lead to a decreased number of dendritic spines [85], for instance, epilepsy, alcoholism, and others disorders, imply that the decline in the number and availability of axo-spinous synapses are the consequence of the dendritic spines loss (85). Previously, our group informed significant dendritic spine loss after ozone inhalation in the

hippocampus, correlated with memory alterations [84], also, dendritic spines loss in the corpus striatum and cerebral cortex with motor impairments [86] as well as olfactory bulb modifications [87]. Furthermore, we found dendritic spine loss in the corpus striatum after V_2O_5 inhalation [8]. Since V interacts with the cytoskeleton, this interaction may be the cause of dendritic spine loss since it seems that actin is a critical element for dendritic spine architecture preservation. It orchestrates the spine's morphology and number [88]. In this context, Pelucchi and cols. [88] mention that Rho activation is essential for the dendritic spine functionality, cofilin phosphorylation, and, consequently, spine actin stabilization. According to Wang et al. [89], cofilin phosphorylation prevents binding to the F- and G-actin binding, and only a dephosphorylated cofilin can initiate the actin-binding. Consequently, their activity is synchronized by phosphorylation/dephosphorylation. It is important to mention again that V is practically a structural and electronic phosphate analog and a phosphatase inhibitor [90]. In humans, the resemblance between phosphate and V explains V and phosphate-dependent enzymes interplay. Therefore, V may achieve a regulatory function in phosphate-depending metabolic processes [90].

It is well known that V neurotoxic properties have been predominantly attributed to its capacity to induce oxidative stress by the generation of ROS, which in turn initiates the peroxidative decomposition of the cellular membranes

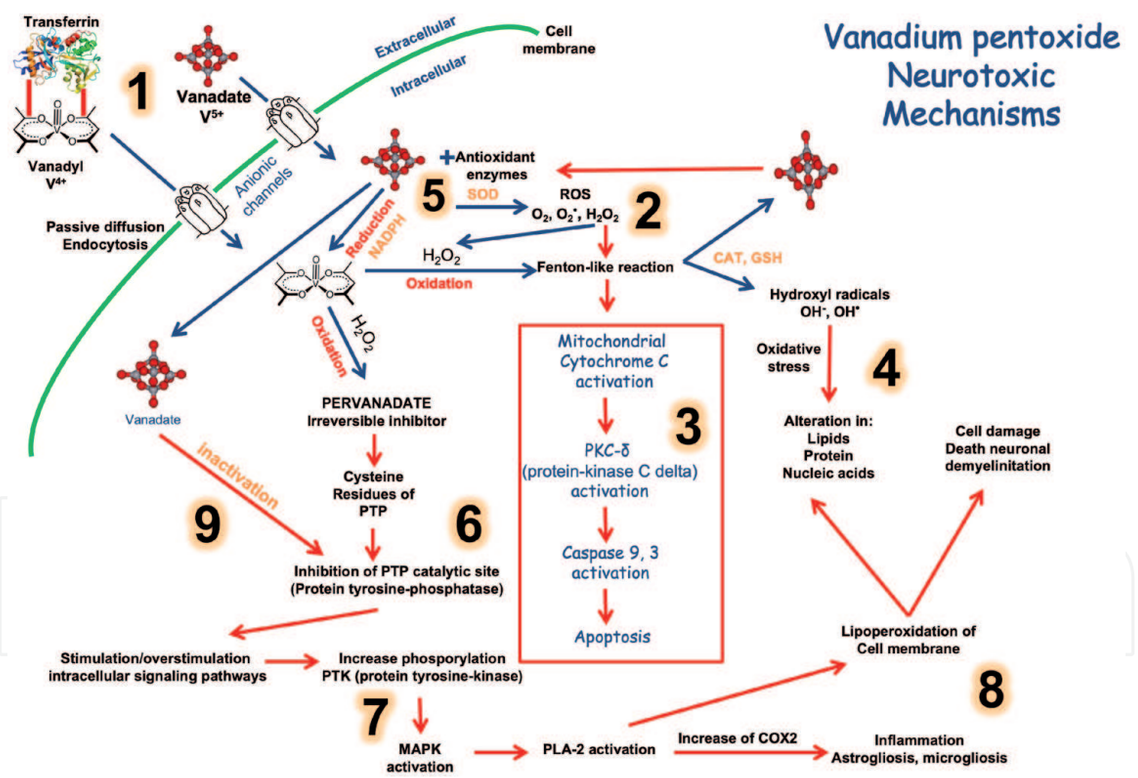

Figure 7.
*When vanadium enters the body, it enters as a tetravalent ((vanadyl) or as a pentavalent (V^{5+}) [3]; then, it is transported via the blood by albumin and transferrin (**1**). V with these two valences enters cells through anionic channels. These two forms arrive the cells through anionic channels; once in the cell, V^{5+} reacts with some antioxidant enzymes such as superoxide dismutase (SOD) (**2**) [12], producing H_2O_2 through Fenton-like reaction, where the mitochondrion initiates the cytochrome C pathway inducing the apoptosis route through the activation of caspases 3 and 9 (**3**) [95], then, vanadate generates free radicals (OH+ OH-) by reacting with GSH and CAT enzymes (**4**) [94], stimulating oxidative stress triggering lipids, proteins, and DNA alterations. V^{5+} reduces to vanadyl through NADPH-oxidase (**5**), which in turn, forms pervanadate, oxidized by H_2O_2, that will permanently inhibit protein tyrosine phosphatases (PTP) [96] (**6**), which will aggregate the phosphorylated protein tyrosine kinase (PTK) activating intracellular signaling pathways (**7**) [1], triggering the inflammation mechanisms through phospholipase-A2 (PLA-A2) and COX-2 formation, activating the gliosis process (**8**) [97], similarly DNA, cell death, demyelination and damage to proteins through lipid peroxidation. Finally, the PTP is inactivated by vanadate (**9**) [98], which results in the activation of intracellular death signaling pathways.*

phospholipids [6, 44, 45] and neuron inflammation [91]. It is also associated with hypomyelination correlated with oxidative stress [92] and a decrease in myelin essential protein [93]. It has also been reported that V produces DNA cleavage, apoptosis and induces iron-mediated oxidative stress in brain cell cultures [94] and hippocampus neuronal death [36]. Likewise, it has been reported that V inactivates protein-tyrosine-phosphatases (PTP) because it binds to the cysteine catalytic residue, which leads to an increase in phosphorylation of PTP, increasing the phosphorylation of the MAPK pathways, which probably causes tau protein hyperphosphorylation, to generate or induce neurofibrillary tangles (NFTs) [94]. Thus, according to our findings and the revised literature, V neurotoxic effects are summarized in **Figure 7**.

Likewise, an increased body of evidence implicates oxidative stress as involved in at least the propagation of cellular injury, which leads to neuropathology in various conditions, such as AD. Moreover, oxidative stress is intimately linked with an integrated series of cellular phenomena, which all seem to contribute to neuronal death [51, 99].

The facts mentioned above provide evidence that V_2O_5 disrupts critical neuronal processes and leads to alterations that include ROS generation, producing cell death. Further work should be done to answer questions, such as identifying the signaling pathways that induced the changes reported here.

Furthermore, as formerly reported, V_2O_5 modifies cytoskeletal proteins such as ɣ-tubulin [54], inducing actin alterations [52]. Some studies have demonstrated the interaction between V with actin. V has a high affinity for cytoskeletal actin-binding sites. G- and F- actin interact with oxovanadium (IV), with 4:1 and 1:1 stoichiometries, respectively, and it has been demonstrated that G-actin-V interaction might occur close to the actin adenosine triphosphate binding position [100–102]. Likewise, decavanadate can modify actin's structure by oxidizing its cysteines in its polymerized form [103].

Remarkably, earlier results demonstrate that V induces Tau hyperphosphorylation [104, 105], ROS, and neuronal inflammation [106], occasioning AD-like damage. Moreover, the substantial hippocampal CA1 cell damage might result from the affinity of G-actin for V, and its association with the metal, since neurons have a particularly dynamic cytoskeleton, which requires continuous polymerization of actin filaments [107].

6. Conclusion

Our results show that vanadium pentoxide, when inhaled, produces important synaptic alterations, manifested in this case, by the significant loss of dendritic spines of CA1 pyramidal neurons and by the presence of Alzheimer-type fibrillar tangles, an aspect considered to be the main neuropathological feature in AD [107], related to the evident alterations of the cytoskeleton. Therefore, more research is needed to establish the relationship between V_2O_5 and Tau hyperphosphorylation, not only in the hippocampus but also in the amygdala, neocortex, and entorhinal, structures involved in AD [108, 109], and whether spatial memory is altered.

Moreover, these data must encourage research efforts towards environmental health effects, with the final purpose of intervening in decrease metals atmospheric pollution such as V. We have to promote viable schemes to safeguard the CNS from toxicants, which have redoubled in the atmosphere during the last decades and represent an important health challenge since metal pollution has been related to neurodegenerative diseases.

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Conflict of interest

The authors declare no conflict of interest.

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