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Vanadium exposure-induced striatal learning and memory alterations in rats

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Abstract

Occupational and environmental exposure to vanadium has been associated with toxicities in reproductive, respiratory, and cardiovascular systems. The knowledge on whether and how vanadium exposure caused neurobehavioral changes remains incomplete. This study was designed to investigate the changes in learning and memory following drinking water exposure to vanadium, and to conduct the preliminary study on underlying mechanisms. Male Sprague-Dawley rats were exposed to vanadium dissolved in drinking water at the concentration of 0.0, 0.5, 1.0 and 2.0 g/L, as the control, low-, medium-, and high- dose groups, respectively, for 12 weeks. The results by the Morris water maze test showed that the time for the testing animal to find the platform in the high exposed group was increased by 82.9% and 49.7%, as compared to animals in control and low-dose groups ($p < 0.05$). There were significantly fewer rats in the medium- and high- dose groups than in the control group who were capable of crossing the platform ($p < 0.05$). Quantitation of vanadium by atomic absorption spectrophotometry revealed a significant dose-dependent accumulation of vanadium in striatum ($r = 0.931$, $p < 0.01$). Histopathological examination further demonstrated a degenerative damage in vanadium-exposed striatum. Interestingly, with the increase of the dose of vanadium, the contents of neurotransmitter ACh, 5-HT and GABA in the striatum increased; however, the levels of Syn1 was significantly reduced in the exposed groups compared with controls ($p < 0.05$). These data suggest that vanadium exposure apparently reduces the animals' learning ability. This could be due partly to vanadium's accumulation in striatum and the ensuing toxicity to striatal structure and synaptic plasticity. Further research is warranted for mechanistic understanding of vanadium-induced neurotoxicity.

Keywords

Vanadium; Striatum; Learning and memory; Neurotransmitter; Synapsin-1

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Declaration of conflict of interest

The authors report no conflicts of interest.

1. Introduction

Vanadium (V) is one of the essential trace elements in human and animal, and participates in many important physiological activities, such as promoting hematopoietic function, reducing blood glucose concentration, protecting the islet cell, lessening atherosclerosis, and performing anti-hyperlipidemic, anti-hypertension or anti-apoptosis functions (Coderre and Srivastava, 2004; Guo et al., 2004; Li et al., 2015). Excessive vanadium in the body, however, can produce toxic effects in respiratory, cardiovascular, urinary, blood and digestive systems (Musk and Tees, 1982; Todaro et al., 1991; Woodin et al., 2000). Over-exposure to vanadium has been shown to cause reproductive and developmental toxicities and even cancer (Domingo, 1996; Ganquli et al., 1994; Ress et al., 2003; Rojas et al., 1999). However, the effect of prolonged exposure to vanadium via the drinking water on the central nervous system remained unclear.

Because of its special physical and chemical properties, vanadium is widely used in petrochemical industry, iron and steel smelting, welding, catalyst, pigment, storage battery, and preservatives (Fortoul et al., 2014; Imtiaz et al., 2015). Vanadium compounds can be absorbed into the body through the respiratory, dermal and/or gastrointestinal pathways during mining and metal production. The discharge of waste gas, residue, and industrial waste water contributes to large amounts of vanadium in the water, soil and air. In our daily life, the combustion of gasoline, coal and other fuels also increases the environmental vanadium load, resulting in vanadium-associated environmental pollution and occupational hazard (Bastami et al., 2015; Imtiaz et al., 2015; Zaytseva et al., 2014). Research by this group on workers who are occupationally exposed to vanadium has shown that vanadium exposure is associated with worker's increased negative mood (Feng et al., 2012a, 2012b). Prolonged exposure for more than 10 years to vanadium among workers can lead to neuronal symptoms such as dizziness, headache, and loss in subjective memory (Li et al., 2013). More recent data suggest that vanadium exposure causes cognitive defects, altered neurobehavioral function, and impaired spatial learning ability (Azami et al., 2012; Mustapha et al., 2014). However, the mechanisms underlying these changes are unknown.

Studies in literature suggest that exposure to vanadium through inhalation can cause damages to medium spiny neurons in the striatum (Avila-Costa et al., 2004). Cumulative evidence has also established that the striatum, as a main component of the basal ganglia, not only participates in the regulation of voluntary movement, but also receives various sensory information and cognitive information, thereby modulating the learning and memory functions (Mu et al., 2010; Packard and Knowlton, 2002). Therefore, it became interesting to learn whether vanadium exposure may result in the accumulation of the metal in the striatal region and how this may lead to the learning and memory alterations.

This study was designed to expose animals to vanadium through the drinking water for a prolonged period and to observe neurobehavioral changes as the function of vanadium accumulation in striatum. In the process, we also attempted to investigate the underlying mechanisms by examining the expression of synapsin-1 (Syn1), a key protein participating in neurotransmitter release and synaptic plasticity, and quantifying the levels of neurotransmitters in striatum. Results of this study will establish the relationship between

vanadium exposure and the learning and memory ability, thereby providing the basis for future study to understand the mechanism by which vanadium causes the neurotoxicity.

2. Materials and methods

2.1. Materials

Chemicals and assay kits were purchased from the following sources: assay kits to quantify ACh, 5-HT and γ -GABA in brain tissues were purchased from Jiancheng Biotech (Nanjing, China); rabbit polyclonal anti-Syn1 antibody (Ab64581) from Abcam (Shanghai, China); biotinylated anti-rabbit antibody IgG (A0208) from Beyotime Biotech (Shanghai, China); antibody against GAPDH, anti-mouse IgG conjugated with horse-radish peroxidase and β -actin were purchased from Beyotime Biotech (Shanghai, China); RIPA buffer (BYL40825) from Jrdun Biotech (Shanghai, China); BCA Assay Kit (PICPI23223) from thermo (Shanghai, China) and all other chemicals were obtained from Jrdun Biotech (Shanghai, China). Tanon-5200, a fluorescence measurement system, was purchased from Tanon (Shanghai, China). All reagents were of analytical grade, HPLC grade or the best available pharmaceutical grade. Video/computer tracking system and MT-200 image analyzing software was purchased from Taimeng Co. (Chengdu, China).

2.2. Animals

Male Sprague Dawley rats were purchased from the Third Military Medical University Animal Experiment Center [License Number: SCXK (Chongqing) 2012-0005]. Upon arrival, animals were housed in a temperature-controlled (25 ± 2) °C, 12-h light/dark cycle room (4 rats per cage) and allowed to acclimate for one week prior to experimentation. At the time use, rats were four weeks old and weighted 56–76 g. Animals had ad libitum access to filtered tap water and pelleted rat chow from the Experimental Animal Center of Zunyi Medical University. The study was approved by the Experimental Animal Ethics Committee of Zunyi Medical University.

Sodium metavanadate was dissolved in drinking water at concentrations of 0, 0.5, 1.0 and 2.0 g/L as the control, low-, medium-, and high- dose groups, respectively. Rats ($n = 32$) were randomly divided into 4 groups; those in V-treated groups were exposed to V in drinking water for 12 weeks. These dose regimens were chosen based on studies reported in literature (Azami et al., 2012; Cui et al., 2012; Zhou, 2007) and also reported by a more recent study (Roberts et al., 2016). Twelve weeks after vanadium exposure, rats were trained for 5 days and tested for learning and memory functions using Morris water maze. All steps of animal training and testing were performed during the dark cycle.

2.3. Behavioral training and testing

Spatial learning ability was tested in the Morris water maze (Morris, 1984). The water maze was a 1.2-m diameter gray-painted cylindrical pool filled with water at 26 °C that contained a 10-cm diameter Plexiglas escape platform hidden 1 cm below the surface of the water. The pool water was made opaque by adding 150 ml of nontoxic white tempera paint. The apparatus was located in an experimental room rich in environmental cues, such as a window, a door, experimental equipment, and tables. Starting at the 12th week of V

exposure, rats were trained for visible (platform with a flag) and invisible (platform) trials. The platform was placed in the second quadrant and rats were gently placed in the pool at the fourth quadrant facing the pool wall at the first trial. After the first trial, rats were randomly placed into any of the three quadrants other than the platform quadrant. The time that it took for rat to find the platform was recorded by an observer. Rats were given 16 training trials (4 times/day for 4 days) during which their latency to find the platform was measured up to a maximum of 120 s. If the animals failed to locate the platform in 120 s, they were manually placed on the platform (in which case 120 s was recorded as the time). After each training, rats were allowed to rest on the platform for 10 s before the trial was repeated. The invisible platform test was performed immediately after the visible platform experiment. On the 5th day, the flag was removed and the trials were repeated as described and the frequency of crossing the original platform was recorded (He et al., 2014; Wei et al., 2009).

Performance in all trials was automatically recorded and measured by a video/computer tracking system and MT-200 image analyzing software (Taimeng Co., China).

2.4. Histopathology

Following neurobehavioral evaluations, animals were decapitated and their brains were removed. One half of the brain was used for histopathological study and the other half of brain was immediately frozen at -80°C for determining metal concentrations and GABA contents. For histopathological study, brain tissues underwent fixation, dehydration and paraffin embedding. The blocks from striatum were cut into 5- μm thick sections and processed for HE staining. The histopathological changes of striatum tissue were examined under the light microscope.

2.5. Atomic absorption spectrophotometry (AAS) analysis

Vanadium concentrations in brain tissues were quantified by a Varian SpectroAA-240Z flameless graphite furnace AAS. The brain tissues were digested with 10 ml of ultrapure concentrated HNO_3 (Sinopharm Chemical Reagent Co., Ltd, AR Select grade) in MARSXpress microwave-accelerated reaction system for 4 h. At the end of digestion, the tubes were placed on an electric heating plate to evaporate nitric acid to nearly dry. The samples were then diluted in 0.5% dilute nitric acid to the final volume of 2 ml. The standard curves were freshly prepared daily in a ranges of 0–10 $\mu\text{g/L}$ in 0.5% dilute nitric acid.

2.6. Determination of neurotransmitter contents in striatum

To understand the effect of vanadium exposure on neuro-transmitters, we used commercially available testing kits to quantify the concentrations of ACh, 5-HT and GABA in striatal tissue. The striatal tissues were thawed at room temperature, and homogenized in 1:1 (mg/ml) ice-cold phosphate buffer solution, pH 7.4. The mixture was centrifuged at 3500 rpm for 10 min; the supernatant was then separated to determine the concentrations of ACh, 5-HT and γ -GABA according to the instruction of the assay kits.

2.7. Western blot analysis of synapsin I (Syn1)

Rat striatal tissues were directly homogenized in the RIPA buffer (BYL40825) with protease inhibitor cocktail to separate and quantify Syn1 levels. The protein concentration was determined using a BCA Assay Kit. Protein extracts were diluted in the loading buffer, loaded onto a SDS-PAGE gel (40 g of protein per lane), electrophoresed, and then transferred to PVDF membranes. The membranes were blocked with 5% fresh-prepared milk-TBST overnight at 4 °C, washed with TBST, and incubated with rabbit polyclonal anti-Syn1 (1:1000) at 4 °C overnight. The membranes were then incubated with biotinylated secondary anti-rabbit antibody (1:1000) and visualized by utilizing enhanced chemiluminescence. GAPDH was also assayed as the loading control by using its antibody (1:1000). Band intensities were quantified and results were reported as a ratio of Syn1 to GAPDH.

2.8. Statistical analysis

All data were expressed as means $\pm$ S.D. If the data were normally distributed, statistical analyses of the differences between groups were carried out by one-way ANOVA followed by LSD (least significant difference) method for multiple comparison (SPSS 18.0). If the data were not in the normal distribution, they were expressed as $M(P_{25}, P_{75})$; the H Kruskal-Wallis test was used to compare the M of the multiple groups. The differences between the two means were considered significant if p values were equal to or less than 0.05.

3. Results

3.1. Neurobehavioral alterations after vanadium exposure

Starting at the 8th week of vanadium exposure, rats in the high-dose group began to show the redness in mouth and nose, reduced diet consumption, drowsiness, slow reaction, decreased activity; some even had diarrhea. Similar symptoms were observed in animals in the medium-dose group starting at the 8th week, while the rats in the low-dose group were largely normal.

Two-way ANOVA analyses of the data from escape latency experiments revealed that there were statistically significant differences among the four experimental groups (Table 1) ($F=6.847$, $p<0.01$). The time for testing animals to find the platform in the high exposed group was increased by 82.9% and 49.7%, as compared to animals in control and low-dose groups ($p<0.05$, Table 1). Rats in the medium-dose group also had the escape latency significantly longer (about 59.6%) than those in the control group ($p<0.05$).

Consequently, the numbers of animals capable of crossing the platform were statistically significantly different among the four groups (Table 1) ($F=6.333$, $p<0.01$). There were significantly fewer rats in the medium- and high- dose groups than in the control group capable of crossing the platform ($p<0.05$).

3.2. Alteration of striatal structure after vanadium exposure

In order to explore whether vanadium-induced learning deficits are associated with structural damage to the striatum, we compared the striatal structures in different vanadium-exposed groups. As showed in Fig. 1, in comparison to the control tissue, the striatal sections from rats receiving vanadium exposure in drinking water for 12 weeks showed a loosened parenchyma, widened cellular lacuna, concentrated cell nuclei, visible hyperchromatism with excessive nuclear staining, and evident vacuole degeneration. Interestingly, these pathological changes became worsened as the vanadium dose increased (Fig. 1B–D).

3.3. Concentrations of vanadium in striatum following oral exposure

To investigate if vanadium accumulated in the striatum as a result of oral exposure, we used the AAS to determine the striatal vanadium concentrations among different groups. Data presented in Table 2 demonstrated that there were statistically significant differences in the vanadium concentrations in striatum of rats among all four study groups ($H = 17.333$, $p < 0.01$). Accumulation of vanadium was dose-dependent ($r = 0.931$, $p < 0.01$). The striatal concentrations of vanadium in the low-, medium- and high- dose groups were about 1.7, 2.3, and 4.3 fold higher than that of the control group, respectively ($p < 0.05$).

3.4. Altered neurotransmitter contents in rat striatum after vanadium exposure

Three primary neurotransmitters were quantified in the striatum in order to explore the possible mechanism underlying neurobehavioral changes as the consequence of vanadium accumulation in the striatum. Data in Table 3 showed an overall statistically significant difference in the striatal contents of ACh, 5-HT and GABA ($H = 24.792$, 25.000 , 32.000 , respectively, $p < 0.01$). Unexpectedly, however, we found that the contents of all three tested neurotransmitters in the striatum were significantly increased following vanadium exposure as compared to controls. The largest increase appeared to be in the GABA content, about 1.78 fold higher in the high-dose group than in the control group ($p < 0.05$).

3.5. Altered expression of Syn1 by western blot analysis

We used the Western blot technique to quantify the expression of Syn1 in striatum of rats with or without vanadium exposure. Results in Fig. 2A demonstrated that the expression of Syn1 was dose-dependently decreased in the vanadium groups as compared to the control. The extent of this decrease was about 40%, 66% and 69% in the low-, medium-, and high-dose groups, respectively, as compared to the control group ($p < 0.05$) (Fig. 2B).

4. Discussion

Results presented in this report clearly indicate that exposure to vanadium in drinking water for 12 weeks results in a significant accumulation of the metal in striatum. Aside from the systemic disorders, such as reduced diet consumption, increased salivation, drowsiness, slow reaction, and overall decreased activity, which could be due to vanadium-induced general systemic toxicity, the most significant observation of this work pertains to vanadium exposure-associated neurobehavioral changes in animals' learning ability. In addition, accumulation of vanadium in striatum causes visible pathological damage to the striatal structure and alters the neurotransmitter contents in striatum.

Aside from its pivotal role in regulating motor functions, the striatum, by receiving the sensory and cognitive information, participates in learning and memory functions (Mu et al., 2010). Yet, cumulative evidences support a role of the basal ganglia, in particular the dorsal striatum, in learning and memory (McDonald and White 1993; McDonald and White 1993; Parkcard and Knowlton, 2002). Thus, vanadium-induced learning deficiency could be partly owing to its detrimental effect on striatal structure. To test this hypothesis, we compared the striatal structure among different vanadium-exposed groups. Data from our histopathological examination clearly showed the structural damage and neuronal apoptosis in the striatum, characterized by edema and vacuole degeneration, after vanadium exposure. Reports in literature also suggest that vanadium can produce free radicals, damage the biological membrane structure, and cause demyelinating (Soazo and Garcia, 2007). By inhalation exposure to vanadium pentoxide, Avila-Costa and co-workers observe the damage not only on dopaminergic neurons in the substantia nigra, but also on medium spiny neurons in the striatum and the pyramidal neurons in hippocampus (Avila-Costa et al., 2004, 2005, 2006). Thus, it is possible that vanadium-associated learning deficit may be due partly to its toxicity on the striatum tissue.

One of the important mechanisms in the formation of learning and memory is synaptic plasticity. The Hebb synapse hypothesis has long proposed that the synaptic plasticity underlies the phenomenon of the effectiveness of information transmission between neurons with the dynamic changes of neural activity (increase or decrease) (Hebb, 1949). The plasticity of synaptic structures is the basis of its functional plasticity. Synaptic proteins, such as Syn1, located in the presynaptic vesicles, are a group of phosphoric acid proteins which, in addition to their recognized function in regulating neurotransmitter release at synapses, can regulate the elongation of axons and dendrites, participate in the formation of synapses, maintain and promote the early development of neurons, and regulate the release of synaptic vesicles (Cesca et al., 2010). After two-month exposure to vanadium in drinking water, the expression level of Syn1 in striatum was significantly decreased in a dose-dependent fashion. We therefore hypothesize that the decreased Syn1 may contribute to the altered synaptic plasticity, which ultimately leads to the learning and memory dysfunction.

A pivotal role of Syn1 pertains its regulation of synaptic release of a variety of neurotransmitters, such as 5-HT, GABA, and ACh. Noticeably, these neurotransmitters actively participate in the transmission, process and storage of the information in the central nervous system and in regulation of the neurobehavioral function. For example, studies in literature suggest that 5-HT abnormality is associated with depression, suicide, obsession and other psychiatric diseases (Liu et al., 2000; Yao, 2006). GABA, a major inhibitory neurotransmitter, has an important effect on learning and memory (Abellán et al., 2000; Giorgetti et al., 2000). Studies have also shown that both muscarinic and nicotinic acetylcholine receptors are involved in encoding of new memories, thus suggesting a critical role of ACh in formation and maintenance of memory and learning (Hasselmo, 2006). All three neurotransmitters are known to present in the striatum (Shu et al., 1990, 1999; Zhang et al., 2000). Since the role of Syn1 is to regulate these neurotransmitters, the reduced expression of Syn1 may be associated with the changes in these neurotransmitters in the striatum; but more research shall be done to verify this point.

Three primary neurotransmitters were quantified in the striatum in order to explore the possible mechanism underlying neurobehavioral changes as the consequence of vanadium accumulation in the striatum. Surprisingly, however, our data show that the levels of all three neurotransmitters tested increased with the increase in vanadium dose. After vanadium poisoning, this overall increase appears to be inconsistent with the mechanism due to changes of neurotransmitter alone. Since the neurotransmitters involved in learning and memory are multifaceted, a single neurotransmitter change is deemed insufficient to explain the mechanism of vanadium neurotoxicity.

In summary, the data presented in this report indicate that long-term vanadium exposure in drinking water can lead to the accumulation of vanadium in the striatum; vanadium accumulation causes striatal tissue damage that may be associated with reduced learning and memory function in animals. Although vanadium exposure resulted in decreased expression of Syn1 and increased neurotransmitters content in the striatum, and this reduction appears to be associated with the overall increase in ACh, 5HT and GABA, the effects of learning and memory are numerous and complex, in addition, the levels of neurotransmitters in a particular brain region may not necessarily be increased or decreased to reflect neuronal malfunctions; the processes associated with their release, uptakes and repackaging could equally affect the normal neuronal activity, may also be associated with prominent proteins, hydrolyzed proteins and multiple receptors. Thus, future work shall be directed toward the understanding of the interaction among the neurotransmitters, their release in synapses and synaptic plasticity with or without vanadium exposure. In conclusion, more in-depth research is needed to explore the mechanism of vanadium-induced neurotoxicity.

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Abbreviations

V	vanadium
Syn1	synapsin-1
AAS	atomic absorption spectrophotometry

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(A: control group, B: Low-V group, C: Medium-V group, D: High-V group, HE, $\times 200$)

Fig. 1.

Histopathological examination of striatum following exposure to vanadium in drinking water for 12 weeks. Brain tissues were treated for HE staining (x200) and typical striatal images are shown in (A) control; (B) low-dose group; (C): medium-dose group; (D) high-dose group. There were dose-dependent loosened parenchyma, widened cellular lacuna, concentrated cell nuclei, and visible hyperchromatism with excessive nuclear staining, Arrows indicate the vacuole degeneration.

Fig. 2.

Expression of Syn1 in striatum of rats by Western-blot analysis. Striatal tissues were dissected from rats receiving vanadium exposure in drinking water for 12 weeks. Tissue homogenates were subjected to Western-blot analysis for Syn1 (74 kD), using GAPDH (37 kD) as the house keeping protein. (A) Data represent mean $\pm$ S.D., $n = 3$, *: $p < 0.05$ compared to controls; #: $p < 0.05$ compared to the low-dose group. (B) Typical image from $n = 3$ images.

Table 1

Effect of vanadium on the learning and memory function of rats (means $\pm$ SD).

Groups	N	Day				Escape latency (s)	Crossing platform
		1d	2d	3d	4d		
Control	8	47.01 ± 22.32	40.08 ± 15.80	34.68 ± 14.25	27.95 ± 13.90	37.43 ± 8.82	4.00 ± 1.69
Low Dose	8	62.20 ± 17.62	49.27 ± 23.83	36.34 ± 9.04	35.02 ± 19.91	45.71 ± 12.44	2.75 ± 0.71
Medium Dose	8	65.12 ± 32.06	74.73 ± 33.76	54.64 ± 36.24	44.47 ± 23.69	59.74 ± 22.91 *	1.75 ± 1.39 *
High Dose	8	73.45 ± 24.83	73.78 ± 11.98 *	66.04 ± 30.01	60.53 ± 28.47 *,#	68.45 ± 12.06 *,#	1.63 ± 0.92 *
F		1.589	4.657	2.916	3.239	6.847	6.333
P		0.214	0.009	0.052	0.037	0.001	0.002

Rats were exposed to vanadium in drinking water at the concentrations of 0.0, 0.05, 1.0, and 2.0 g/L as control, low-dose, medium-dose and high-dose groups, respectively. Data represent Mean $\pm$ S.D.

^{*} $p < 0.05$ compared with controls.

[#] $p < 0.05$ compared with the low-dose group.

Table 2Determination of v content in striatum [M (P₂₅, P₇₅)].

Groups.	N	V content(ng/g pro.)
Control	6	4.07(3.35, 5.24)
Low Dose	6	6.99(6.27, 7.77) *
Medium Dose	6	9.53(8.85, 9.87) *
High Dose	6	17.53(13.79, 19.26) *,#, &
<i>H</i>		17.333
<i>P</i>		0.001

Please see the legend to Table 1 for animal treatment.

*
p<0.05 compared with controls.#
p<0.05 compared with the low-dose group.&
p<0.05 compared with the medium-dose group.

Table 3

Neurotransmitter content in striatum of rats.

Groups	N	ACh(pmol/L)	5-HT(ug/g pro.)	GABA(mg/g pro.)
Control	8	161.62 ± 14.31	2.63(2.59, 2.71)	0.14(0.13, 0.14)
Low Dose	8	185.07 ± 9.51 *	3.35(3.21, 3.38) *	0.19(0.19, 0.20) *
Medium Dose	8	191.27 ± 5.98 *	3.82(3.64, 3.97) *,#	0.22(0.21, 0.24) *,#
High Dose	8	202.83 ± 7.60 *,#,&	4.29(4.15, 4.51) *,#,&	0.25(0.25, 0.26) *,#,&
<i>H</i>		24.792	25.000	32.000
<i>P</i>		0.001	0.001	0.001

Please see the legend to Table 1 for animal treatment.

* $p < 0.05$ compared with controls.# $p < 0.05$ compared with the low-dose group.& $p < 0.05$ compared with the medium-dose group.