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The Peroxidative Changes in Rat Brain Tissue Homogenates by Vitamin C and Deferoxamine against Vanadium-Stimulated Lipid Peroxidation

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Abstract

Objectives Vanadium (V) metal induces lipid peroxidation (LPO) and this has been proposed as a cause for its neurotoxicity.

Aim This study aimed to evaluate the effects of vitamin C (VC) and deferoxamine (DF) against V-induced LPO in brain tissue homogenates in vitro.

Methods Male Sprague-Dawley rats were used. Brains were removed and dissected into hypothalamus, hippocampus, brain stem, and medulla pons. They were homogenized in150mM potassium chloride (KCl), and incubated for 1 hour with V, VC, and DF in a micromolar concentration of 20 and 100. Aliquots were used for the estimation of LPO in spectrophotometer. Data analysis were done by one-way analysis of variance.

Results V exposure (20 and 100μM) demonstrated statistically significant ($p < 0.001$) enhancement of LPO (average increase with 20μMV was by +105% and with 100μMV was by +130%), respectively, in brain tissue homogenates compared with water controls. Hypothalamus exhibited maximum enhancement (average increase with 20μMV was by +145% and with 100μMV was by +175%, respectively), in LPO than other regions. Coexposure of brain tissue homogenates to V + VC (20 and 100μM) further accelerated the LPO (+24% and +16%, respectively) compared with V alone. Brain stem exhibited highest increases (+54% with 20μMV and +21% with 100μMV; $p < 0.001$), respectively. V-induced oxidative consequences were remarkably inhibited (average -55%; $p < 0.001$) by DF + V (20μM + 100μM) exposure. Hypothalamus and medulla pons exhibited inhibition, by −66% and −60% ($p < 0.001$) respectively.

Conclusion V exposure in vitro resulted in oxidative damage with significant regional variations in brain tissue homogenates. VC is pro-oxidative in vitro reactions and DF chelates V-ion moiety.

Keywords
- brain tissues
- deferoxamine
- homogenates
- lipid peroxidation
- vanadium
- vitamin C

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ملخص المقال باللغة العربية

التغيرات البيروكسيدية لفيتامين ج وديفيروكسامين في أنسجة دماغ الفئران ضد بيروكسيد الدهون المحفزبواسطة الفاناديوم

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الخلفية: بحث معدن الفاناديوم على أكسدة الدهون، وقد تم اقتراح هذا التأثير كسبب أساسي لسميته على الجهاز العصبي.

الأهداف: هدفت هذه الدراسة إلى تقييم تأثيرات فيتامين ج وديفيروكسامين ضد أكسدة الدهون المستحث بـ فانيديوم في متجانسات أنسجة المخ في المختبر.

المواد والطرق: تم استخدام ذكور الجرذان من نوعية السبراغ دولي. تمت إزالة الأدمغة وفصل كل من تحت المهاد، والحصين، وجذع الدماغ، والنخاع المستطيل والجسر. تم تخليط العينات في 150 ملي مول من كلوريد البوتاسيوم، وحضنت مع الـ فانيديوم لوحدة أو الفانيديوم مع فيتامين ج أو ديفيروكسامين لمدة ساعة واحدة. تم إجراء ثلاث سلاسل من التجارب. السلسلة الأولى، المتجانسات كانت محضنة بعنصر الفاناديوم (20 أو 100 ميكرومول). السلسلة الثانية، المتجانسات كانت محضنة بفيتامين ج (20 أو 100 ميكرومول) بالإضافة إلى الفاناديوم (20 أو 100 ميكرومول). السلسلة الثالثة، المتجانسات محضنة بديفيروكسامين (20 أو 100 ميكرومول) بالإضافة إلى الفاناديوم (20 أو 100 ميكرومول). تم استخدام المحاليل لتقدير درجة أكسدة الدهون عن طريق قياس مستويات والمالونديالدهيد باستخدام مقياس الطيف الضوئي.

التحليل الإحصائي: تضمن تحليل البيانات تقدير متوسط ± الانحراف المعياري، ومقارنة المجاميع باستخدام طريقة واحدة لتحليل التباين واختبار (ت) لعينتين مرتبطتين. تم تحديد مستوى الدلالة الإحصائية عند$p <0.05$.

النتائج: التعرض لـ فانيديوم (20 و100 ميكرومول) عزز أكسدة الدهون في متجانسات أنسجة المخ مقارنة مع المجموعة المرجعية (متوسط + 105 ٪ و + 130 ٪ على التوالي). أظهر المهاد أعلي التغيرات في أكسدة الدهون مقارنة بالمناطق الأخرى (+ 145٪ و + 175٪ على التوالي). أدى التعرض المشترك لأنسجة المخ إلى الـ فانيديوم بالإضافة إلى فيتامين ج (20 و100 ميكرومول) إلى تسريع أكسدة الدهون مقارنة بـ فانيديوم وحده (+ 24٪ و + 16٪ على التوالي). أظهر جذع الدماغ أعلي التغيرات (+ 54٪ و + 21٪ على التوالي). قام ديفيروكسامين (20 ميكرومول + 100 ميكرومول) بتثبيط أكسدة الدهون التي يسببها فانيديوم بشكل ملحوظ (متوسط -55 ٪). كان التثبيط أعلي في تحت المهاد والنخاع المستطيل والجسر (-66٪ و -60٪) على التوالي.

الاستنتاج: أدى تعرض أنسجة المخ لـ فانيديوم في المختبر إلى تلف مؤكسد مع اختلافات إقليمية كبيرة. كان الفيتامين ج معزز للأكسدة في التفاعلات المختبرية، أما ديفيروكسامين فله تأثير مثبط نتيجة إستخلاب أيوني لـ فانيديوم.

الكلمات المفتاحية: الفاناديوم، فيتامين ج، ديفيروكسامين، بيروكسيد الدهون، أنسجة المخ، المتجانسات، ضرر بيروكسيدي.

Introduction

Vanadium is a ubiquitous element. The use of vanadium due to its insulin-mimetic properties has been referred to as antidiabetic agent and anabolic agent.[1] On the other hand, environmental and occupational vanadium exposure has been associated with several deleterious health hazards linked with carcinogenic, immunotoxic, and neurotoxic insults.[1] Among the handful of proposed mechanisms of vanadium toxicity, which include interference with lipids, and proteins, depletion of antioxidant defense system and induces double-strand breaks in DNA. However, among all induction of oxidative stress is of paramount importance for biological systems.[2]

We have established that vanadium induces lipid peroxidative damage and this has been proposed as a likely basis for its neurotoxicity.[3–6] Antioxidants such as vitamin E, vitamin C, selenium, and doxycycline have been used with success against vanadium toxicity in experimental animals.[3–7]

The literature review provides in vitro only one such study,[1] where manganese decreased the cellular uptake of vanadium and prevented DNA damage in hepatocytes.[1] The general view is that vanadium has the potential to induce aneuploidy, micronucleus, and chromosomal aberrations in some cells in vitro and in vivo.[8]

Therefore, the aim of this study was to examine in vitro effects of vanadium on occurrence of lipid peroxidation (LPO) in fresh homogenates in rat brain hypothalamus, hippocampus, mid-brain, and medulla-pons, to identify possible beneficial effects of vitamin C (ascorbic acid) and deferoxamine, which is a chelator class of drug for iron and aluminum toxicity),[9] against vanadium-stimulated lipid peroxidative damage.

Methods

Chemicals

Sodium metavanadate, L-ascorbic acid, trichloroacetic acid, and 1,1,3,3 tetraethoxy-propane were purchased from Sigma Chemical Co. (St. Louis, Missouri, United States). Deferoxamine was obtained from Ciba-Geigy AG (Klybeckstrasse 141, Basel, 4002 Switzerland) and potassium chloride was purchased from Merck Darmstadt (Federal Republic of Germany).

Operative and Sampling Technique

Standard anatomical technique for the dissection of multiple brain regions from a single brain and standard biochemical colorimetric procedures were utilized in this study.

All experiments were performed on brain hippocampus, hypothalamus, mid-brain, and medulla-pons tissues from fed six male Sprague-Dawley rats (body weight 300–400 g) obtained from the Central Animal House of the Faculty of Medicine, University of Benghazi. Animals were sacrificed by decapitation without anesthesia. The brains were rapidly excised on a petri dish placed on crushed ice, and the tissues were kept at −80°C until used for the in vitro experiments.

Preparation of Brain Homogenates

Brain tissues, hippocampus, hypothalamus, mid-brain, and medulla pons (up to 400 mg from each brain) were dissected on an ice plate and transferred to an ice-cold 150 mM potassium chloride solution. The tissue was kept in the cold medium for 10 minutes before it was homogenized in chilled 150 mM KCl, using a glass homogenizer with Teflon pestle fitted to motor drive, and the volume was adjusted to give a 10% w/v homogenate. In this study, brain tissue homogenates were exposed to elemental vanadium in the form of sodium metavanadate. The chosen exposure levels (20 µM and 100µM) were referred to as no observed adverse effect level concentration. Previously, one research group has used the same concentration(in µM) in an oral study[10] therefore, equivalent concentration of antioxidants vitamin C as ascorbic acid (20 and 100 µM) and deferoxamine (20 and 100 µM) was added to the reaction mixture. Three series of experiments were performed. In the first series, homogenates were equilibrated under molecular oxygen on bench followed by addition of elemental vanadium (20 and 100µM). In the second series, homogenates were equilibrated with molecular oxygen on bench followed by addition of vitamin C (20 and 100 µM) + vanadium (20 and 100 µM). In the third series, homogenates were equilibrated with molecular oxygen on bench followed by addition of deferoxamine (20 and 100 µM) + vanadium (20 and 100 µM).

Determination of Malondialdehyde Levels

The content of malondialdehyde (MDA) formation (a product of LPO) was estimated in the form of thiobarbituric acid reactive material. It was estimated in four sets of homogenate reaction mixtures, namely control, vanadium exposed, vitamin C + vanadium tested, and vanadium + deferoxamine tested. Aliquots (200 µL), containing 300–400 mg brain tissue homogenates (in triplicate) [11] were pipetted in test tubes followed by addition of 200 µL of 8.1 of sodium dodecyl sulfate, 1.5 mL of 20% acetic acid, and 1.5 mL of 1% thiobarbituric acid. The contents were mixed on a vortex mixer. The final volume of the reaction mixture was adjusted to 4 mL with distilled water. The tubes were mixed on a vortex mixer. The samples were heated in a hot water bath at 100°C for 60 minutes. After cooling under tap water, the samples were added 1 mL of distilled water, extracted with 5 mL of *n*-butanol: pyridine (15 mL: 1 ml v/v), mixed vigorously on a vortex mixer, and centrifuged at 4,000 rpm for 15 minutes. The MDA content in the *n*-butanol: pyridine layer was then spectrophoto-metrically determined at 535 nm.[12] MDA levels were calculated from the standard curve using 1, 1, 3, 3-tetra ethoxy propane and expressed as nano-moles of MDA/g fresh tissue.

Statistical Analysis

The data was presented as means ± standard error of mean ($n = 6$). Data were analyzed by one-way analysis of variance. When the analysis indicated a significant difference ($p < 0.05$), the fortified groups were compared with their respective controls. Statistical analysis was performed by F-test for homogeneity of variance followed by *t*-test.[13]

Results

In Vitro Effect of Vanadium on Lipid Peroxidation in Homogenates from Different Regions of Rat Brain

The results in ►**Table 1** demonstrate a dose–response relationship in the occurrence of LPO following exposure to vanadium (20 and100 µM). The order of enhancement was hypothalamus (+145 and 175%), hippocampus (+111 and 126%), mid-brain (+81 and 113%), and the medulla-pons (81 and 325%, respectively (see ►**Table 2**). The hypothalamus was most vulnerable region, where occurrence of LPO was highest. ►**Table 2** shows that the average vanadium (20µM)-induced percent increase in occurrence of LPO was +105% among brain regions compared with the controls. However, the average percent increase in occurrence of LPO in total brain homogenates-induced by vanadium (100µ) was by +185.3%. This was 80.3% faster than in homogenates exposed to 20µM vanadium.

In Vitro Effect of Coexposure to Vitamin C and Vanadium on Lipid Peroxidation in Homogenates from Different Regions of Rat Brain

►**Table 1** and **2** demonstrate that the combined exposure to vitamin C (20 and 100 µM) and vanadium (20 and 100 µM) resulted in dose-dependent provocation in the occurrence of LPO. The average percent increase in LPO in total brain region homogenates was +23.7% compared with homogenate exposed to 20µM vanadium only. This provocation was significant in the brain stem (+54%) followed by medulla pons (+32%). There were non-significant increases in LPO in both the hippocampus (+ 7%) and hypothalamus (+ 2%), respectively. On the other hand, the average percent increase in LPO in total brain homogenates was faster (+17.8%) when compared with brain tissue homogenates exposed to 100 µM vanadium only. The order of

Table 1 The effect of vitamin C and deferoxamine in the rat brain tissue homogenates against vanadium-induced lipid peroxidation

In vitro test	Dose (μ mM)	Brain regions				Total levels of MDA
		Mid-Brain	Hypothalamus	Hippocampus	Medulla-pons	
Lipid peroxidation (nano-mole MDA/fresh weight tissue)						
Control (water)	0	0.471 ± 0.007	0.471 ± 0.090	0.515 ± 0.075	0.458 ± 0.009	1.915
Vanadium	20	0.854[a] ± 0.034	1.156[a] ± 0.039	1.090[a] ± 0.018	0.828[a] ± 0.006	3.928
Vanadium	100	1.007[a] ± 0.018	1.294[a] ± 0.066	1.166[a] ± 0.057	1.950[a] ± 0.030	5.417
Vitamin C + vanadium	20 + 20	1.317[b] ± 0.008	1.179 ± 0.033	1.164 ± 0.042	1.093[b] ± 0.045	4.753
Vitamin C + vanadium	100 + 100	1.447[c] ± 0.002	1.323 ± 0.024	1.279 ± 0.021	1.326[c] ± 0.043	5.375
Deferoxamine + vanadium	20 + 20	0.373[b] ± 0.014	0.398[b] ± 0.018	0.366[b] ± 0.060	0.282[b] ± 0.033	1.419
Deferoxamine + vanadium	*100 + 100*	*0.494[c] ± 0.030*	*0.587[c] ± 0.024*	*0.518[c] ± 0.037*	*0.381[c] ± 0.028*	1.980

Abbreviations: MDA, malondialdehyde; SD, standard deviation.
In vitro test homogenates were incubated in test tubes in hot water bath for 1 hour at 100°C.
Each value represents the mean ± SD from six independent experiments.
[a]$p < 0.001$ versus control group.
[b]$p < 0.001$ versus (20 μM) vanadium group.
[c]$p < 0.001$ versus (100 μM) vanadium group.

provocation was significant both in brain stem (+21%) and medulla-pons (+ 21%), respectively. However, there was non-significant increase in occurrence of LPO in both hypothalamus (+12%) and hippocampus (10%).

In Vitro Effect of Coexposure to Deferoxamine and Vanadium on Lipid Peroxidation in Homogenates from Different Regions of Rat Brain

►**Table 1** and **2** demonstrate that the combined exposure to deferoxamine (20 and 100μM) and vanadium (20 and 100 μM) to rat brain tissue homogenates resulted in significantly dose-dependent inhibition in the occurrence of LPO. The average percent inhibition in total brain region homogenates was by −64% when compared with brain homogenates exposed to vanadium (20μM) only. The sequence of inhibition was alike in hypothalamus (−66%), hippocampus (−66%), and medulla-pons (−66%) followed by mid-brain (−56%). On the other hand, exposure of fresh homogenates from different regions of the brain to deferoxamine (100 μM) and vanadium (100 μM) also exhibited significantly inhibited occurrence of LPO when compared with the brain tissue homogenates incubated to vanadium (100μM) vanadium only. The following was the sequence of inhibition: medulla-pons (−60%), hippocampus (−56%), hypothalamus (−55%), and brain-stem (−51%). The mean percent inhibition of LPO in total brain region homogenates was −56%.

Discussion

Effect of Vanadium (20 and 100μM) on Occurrence of Lipid Peroxidation in Brain Tissues Homogenates

The treatment of brain tissue homogenates with vanadium exhibited dose-dependent significantly enhanced occurrence of LPO compared with water controls. The present results are in perfect congruence with a previous research finding[14] with the trends of enhanced LPO in brain microsomes exposed to 100μM vanadium. It is thus likely that pentavalent (V^{5+}) salt of vanadium, used by us, was reduced to tetravalent (V^{4+}) vanadium by a one electron transfer reaction; therefore, O_2 is reduced to superoxide anion radical (O_2^{-}.).[15] Henceforth, LPO was enhanced in total brain region tissue homogenates.

Effect of Coexposure to Vitamin C (20 and 100 μM) and Vanadium (20 and 100μM) on Occurrence of Lipid Peroxidation in Brain Tissues Homogenates

Our results established that treatment of brain tissue homogenates with a combination of vitamin C (20 or 100 μM) and vanadium (20 or 100 μM) significantly provoked acceleration in the occurrence of LPO compared with exposure of brain tissues with 20 or 100 μM vanadium, respectively. There is an extensive evidence that vitamin C, chemically known as ascorbic acid, has pro-oxidative abilities in the presence of oxygen and transition metal ions under conditions of high millimolar concentration in vitro.[16] Our results can be explained by the reactions that might have triggered the reduction in pentavalent (V^{5+}) to tetravalent vanadium (V^{4+}) along with ascorbate. This process may have increased the generation of reactive oxygen species in Fenton-like redox cycling reaction in the brain homogenates, and enhanced occurrence of LPO.

$$AH_2 \rightarrow AH^- + H^+$$

Ascorbic Acid Ascorbate anion

$$AH^- + V^{5+} \rightarrow A\cdot + V^{4+} + H^+$$

Ascorbate anion Pentavalent vanadium Ascorbyl radical Tetravalent vanadium

$$H_2O_2 + V^{4+} \rightarrow V^{5+} + OH\cdot + OH^- \text{ (Fenton Reaction)}$$

Hydrogen peroxide Tetravalent vanadium Hydroxyl radical Hydroxyl anionl

$$V^{4+} + O_2 \text{ (oxidation)} \rightarrow V^{5+} + O_2^{\cdot -}$$

Table 2 The percentage changes in the rat brain tissue homogenates following exposure to vitamin C and deferoxamine against vanadium-induced lipid peroxidation

In vitro test	Dose (μM)	Brain regions								Total % change in brain regions	
		Mid-brain		Hypothalamus		Hippocampus		Medulla-pons			
		% change from control	% change from vanadium alone	% change from control	% change from vanadium alone	% change from vanadium control	% change from vanadium alone	% change from control	% change from vanadium	% change from control	% change from vanadium alone
Control (water)	0	0	–	–	–	–	–	–	–		
Vanadium	20	81	–	145	–	112	–	81	–	105.1	
Vanadium	100	114	–	175	–	126	–	326	–	185	
Ascorbic acid + vanadium	20 + 20	180	54	150	2	126	7	139	32	148.0	21.0
Ascorbic acid + vanadium	100 + 100	207	43.7	181	2.24	148	10	190	21	180.7	17.8
Deferoxamine + vanadium	20 + 20	−21	−56	−15	−66	−29	−66	−38	−66	−25.9	−64
Deferoxamine + vanadium	100 + 100	5	−51	25	−55	1	−56	−17	−65	3.4	−56.6

This is one electron transfer reaction, where molecular oxygen is reduced to superoxide anion ($O_{2-.}$) and tetravalent vanadium (V^{4+}) is oxidized to pentavalent vanadium (V^{5+}).

Effect of Coexposure to Deferoxamine (20 and 100μM) and Vanadium (20 and 100μM) on Occurrence of Lipid Peroxidation in Rat Brain Tissues Homogenates

It has been evaluated by thiobarbituric acid colorimetric procedure that deferoxamine has the ability to revert LPO.[17] The present results also demonstrated that exposure of brain tissue homogenates to a combination of deferoxamine (20 or 100 μM) and vanadium (20 or 100 μM) significantly inhibited the occurrence of LPO. A similar report by a group of researchers demonstrated that deferoxamine protected against generation of reactive oxygen species following methyl mercury intoxication in rat brain. Our results are in congruence with these authors.[17] Henceforth, in discussing the present results, it seems justified that deferoxamine protected against vanadium-induced occurrence of LPO in brain tissue homogenates via potentiating a very strong affinity chelation with vanadium metal.

Further experiments are required to lend a better insight into the etiopathogenesis of vanadium by using various fortifiers and evaluation of antioxidant enzymes in brain tissue homogenates in rats.

Conclusion

In vitro results demonstrated significant acceleration of LPO in brain tissue homogenates following vanadium exposure. Exposure to vitamin C + vanadium provoked LPO, while deferoxamine + vanadium inhibited the LPO. The brain regional heterogeneity in occurrence of LPO is because the glial cells in various central nervous system sites are not same.[18]

The Limitation of This Study

The present investigation should have included measurement of antioxidant enzymes in brain tissues as a further extension of this study.

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Conflict of Interest

None declared.

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