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Doxycycline: An Antibiotic Attenuates Oxidant Stress, Perturbation of Lipid Metabolites, and Antioxidants against Vanadium Toxicity in Rat Hepatocytes

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Abstract

Background The liver is target following exposure to pentavalent vanadium (V^{5+}). Doxycycline is an antioxidant that prevents the progression of disease through inhibition of lipid peroxidation.

Aim The present study was designed to evaluate the protective effects of doxycycline against vanadium-induced hepatoxicity.

Methods Sixty two male Sprague-Dawley rats (250–300 g) were equally divided into the following four groups: control group (received 0.2 mL of physiological saline), doxycycline control group (received 4 mg/kg body weight on day 1 and 2 mg/kg body weight daily thereafter), vanadium group (received elemental vanadium 1.5 mg/kg-body weight in distilled water), and concomitantly treated group (doxycycline + vanadium) received (doxycycline 4 mg/kg body weight on day 1 and 2 mg/kg body weight thereafter + vanadium 1.5 mg/kg body weight), all given orally for 10 consecutive days. The rats were sacrificed by decapitation 24 hours after the last dose. The liver was removed rapidly and processed for the evaluation of metabolic variables: phospholipids, cholesterol, cerebrosides, gangliosides, reduced glutathione (GSH), vitamin C, calcium, acetylcholinesterase enzyme, and lipid peroxidation.

Results Vanadium administration significantly reduced (−60 g) the body weight and significantly increased (+28%) the relative liver weight compared with controls. The rats exhibited neurological function deficits. Vanadium administration decreased the concentrations of metabolic variables compared with controls, cerebrosides (−50%), cholesterol (−39%), phospholipids (−18%), GSH (−45%), and inhibited acetylcholinesterase enzyme

Keywords
- vanadium
- doxycycline
- lipid profiles
- lipid peroxidation
- glutathione
- ascorbic acid
- acetylcholinesterase

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(–48%). Gangliosides (+ 38%), vitamin C (+ 20%), and calcium (+ 80%) were increased together with an enhancement (+64%) in lipid peroxidation. The combined treatment (vanadium and doxycycline) significantly increased (+25 g) the body weight and relative liver weight of rat was significantly reduced (+5%) compared with vanadium administered group. The levels of metabolic variables were significantly reversed in this group in the following order: cholesterol (+17%), phospholipids (+7%), vitamin C (−14%), acetylcholinesterase enzyme activity (−27%) together with inhibition (−16%) of lipid peroxidation. All levels were ($p < 0.05$). Doxycycline presented no effect on the levels of GSH, cerebrosides, and gangliosides.

Conclusion Results of this study suggested vanadium-induced oxidation of lipids and sphingolipids in hepatocytes and much of GSH was consumed against high production of reactive oxygen species. Doxycycline protected against vanadium-induced oxidative damage that could be attributed to its free radical scavenging effects on membrane-bound lipids and acetylcholinesterase enzyme.

ملخص المقال باللغة العربية

الدوكسيسيكلين: مضاد حيوي يخفف من إجهاد الأكسدة واضطراب الأيض الدهني ومضادات الأكسدة ضد سمية الفاناديوم في خلايا الكبد في الفئران

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الخلفية: الكبد هو الهدف بعد التعرض للتسمم بواسطة الفاناديوم خماسي التكافؤ (V5+). الدوكسيسيكلين مضاد للأكسدة ويمنع تطور التسمم من خلال تثبيط بيروكسيد الدهون.

الأهداف: صممت الدراسة الحالية لتقييم التأثيرات الوقائية للدوكسيسيكلين ضد السمية الكبدية التي يسببها الفاناديوم.

المواد والطرق: تم تقسيم اثنين وستين من ذكور جرذان سبراغ-دولي (250-300 جم) بالتساوي إلى المجموعات الأربع التالية: المجموعة الضابطة (تم تلقي 0.2 مل من المحلول الملحي الفسيولوجي)، مجموعة التحكم دوكسيسيكلين (تم تلقي 4 مجم/كجم من وزن الجسم في اليوم الأول و2 مجم/كجم من وزن الجسم يوميًا بعد ذلك)، مجموعة الفاناديوم (تم تلقيها عنصر الفاناديوم 1.5 مجم/كجم من وزن الجسم في الماء المقطر)، والمجموعة المعالجة بشكل متزامن (الدوكسيسيكلين + الفاناديوم) المتلقاة (الدوكسيسيكلين 4مجم/كجم من وزن الجسم في اليوم الأول و 2مجم/كجم من وزن الجسم بعد ذلك + الفاناديوم 1.5مجم/كجم من وزن الجسم). أعطيت جميعها عن طريق الفم لمدة 10 أيام متتالية. تم قتل الفئران بقطع الرأس بعد 24 ساعة من آخر جرعة. تمت إزالة الكبد بسرعة ودراسة وتقييم المتغيرات الأيضية لكل من: الفسفوليبيدات، الكوليسترول، سيريبروزيد، الغانغليوزيدات، الجلوتاثيون المختزل، فيتامين سي، الكالسيوم، إنزيم استيل كولينستريز ، و بيروكسيد الدهون.

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

النتائج: أدى الفاناديوم إلى انخفاض معنوي (60 جم) من وزن الجسم وزيادة معنوية (28٪) في الوزن النسبي للكبد مقارنة بالمجموعة الضابطة. أظهرت الحيوانات قصورًا في الوظيفة العصبية. خفض الفاناديوم تراكيز المتغيرات الأيضية مقارنة بالمجموعة الضابطة لكل من السيريبروزيد (−50٪) ، والكوليسترول (-39٪) ، الفسفوليبيدات (−18٪) ، و الغلوتاسيون (-45٪). كما تم تثبيط إنزيم الأستيل كولينستريز (- 48٪). وزيادة كل الغانغليوزيدات (+ 38٪) ، و فيتامين سي (+ 20٪) ، والكالسيوم (+ 80٪) مع زيادة (+ 64٪) في بيروكسيد الدهون. أما العلاج المشترك (الفاناديوم و الدوكسيسيكلين) فإنه سبب زيادة معنوية (+25 جم) في الوزن وانخفاض الوزن النسبي للكبد (+ 5٪) مقارنة بالمجموعة المعالجة بواسطة الفاناديوم، كما تم عكس مستويات المتغيرات الأيضية بشكل كبير في هذه المجموعة بالترتيب التالي: الكوليسترول (+ 17٪)، الفوسفوليبيد (+ 7٪) ، فيتامين سي (-14٪) ، نشاط إنزيم أستيل كولينستريز (-27٪) مع التثبيط (- 16٪) من بيروكسيد الدهون. كانت جميع هذه التغيرات ذات دلالة إحصائية عند مستويات ($P<0.05$). ومن ناحية أخري لم يسبب العلاج بالدوكسيسيكلين أي تأثير على مستويات سيريبروزيد، الغانغليوزيدات، والجلوتاثيون المختزل.

الاستنتاج: اقترحت النتائج أن الفاناديوم سبب زيادة في أكسدة الدهون في خلايا الكبد وأن تركيز عال من الغلوتاسيون تم استهلاكه ضد إنتاج عالٍ من أنواع الأكسجين التفاعلية. يحمي الدوكسيسيكلين من التلف التأكسدي الناجم عن الفاناديوم والذي يمكن أن يعزى إلى إزالة الجذور الحرة على الدهون المرتبطة بالغشاء وإنزيم أستيل كولينستريز.

الكلمات المفتاحية: الفاناديوم، الدوكسيسيكلين، تكوين الدهون، بيروكسيد الدهون، الجلوتاثيون، حمض الأسكوربيك، أسيتيل كولينستريز.

Introduction

Vanadium, a transient metal, occurs as a natural component of earth's crust, such as various minerals, coal, and crude oil. Vanadium is released in the environment mainly due to human activities.[1] Toxicity of pentavalent vanadium (V^{5+})—metavanadate salt—is a challenging problem. Exposure to (V^{5+}) is recognized as an industrial hazard that affects humans and animal health. Some researchers have demonstrated that (V^{5+}) solutions induced DNA damage in mice and rats in the following order: liver > heart > kidney.[2] Liver has been recognized as one of the tissues for (V^{5+}) toxicity.[3,4] Several studies have implicated (V^{5+}) toxicity in the formation of reactive oxygen species (ROS), probably by interacting with mitochondrial redox centers.[5–7] It is well established that the reduced glutathione(GSH)-related thiols are involved in many important biological reactions, including the protection of cell membrane against oxidative damage. Furthermore, GSH can reduce (V^{5+}) to (V^{4+}) in most mammalian tissues.[1]

To date, pathways of ROS and perturbations in lipid metabolism in (V^{5+})-induced liver toxicity have not been comprehensively studied.[8] Currently, the preferential pharmacological treatment of metal poisoning is based on chelating therapy because it facilitates removal of metal from soft tissues and excretion in urine.[1] Doxycycline is an antibiotic and a chelating agent being used in the management of several diseases characterized by chronic inflammation.[9] One potential mechanism by which it inhibits the progression of these diseases is by reducing oxidant stress, thereby inhibiting subsequent lipid peroxidation and inflammatory responses. So, in this presentation, we evaluated the therapeutically potential effects of doxycycline, if any, on the ROS, perturbations in lipid metabolism, levels of GSH, and vitamin C, calcium concentration and activity of acetylcholinesterase (AChE) enzyme in the rat hepatocytes following exposure to vanadium.

Materials and Methods

Chemicals

Acetylthiocholine and 1,1,33-tetraethoxypropane were purchased from Sigma Chemical Co., St. Louis, Missouri, United States. Doxycycline hydrochloride was obtained from (Sarvi Pharm., Algeria, Guelma, Vilaya De Guelma, 24000, UK). Sodium metavanadate and all the other chemicals were purchased from BDH chemicals and were of analytical grade.

Animals

Sixty-two male Sprague-Dawley rats weighing between 250 and 300 g were obtained from the Central Animal House of the Faculty of Medicine, University of Benghazi, and were housed in stainless steel cages and adopted to laboratory conditions (25 ± 2°C, relative humidity 40–60%, and 12-hour light-dark cycle). The rats were allowed free access to laboratory pellet diet (National Company of Animal Feeds, Benghazi, Libya) and fresh water ad libitum. The study protocol was approved by the Animal Care and Use Research Ethics Committee, University of Benghazi, Libya.

Mode of Administration

Drugs were given to the rats by oral intubation by means of a small feeding tube through the mouth into the stomach. Distilled water was used as a vehicle for both vanadium and doxycycline. Drugs were given in volumes ranging between 0.25 and 0.30 mL/oral route.

Experimental Groups

The rats were randomly distributed into four experimental groups of 15 rats each. The details were as follows.

Group 1: Saline-control group received 0.2 mL of physiological saline orally for 10 consecutive days.

Group 2: Doxycycline-treated controls received doxycycline (4 mg/kg body weight on day 1 followed by 2 mg/kg body weight hereafter) orally for 10 consecutive days. The subantibiotic dose (low-dose doxycycline) has been widely used in children and in related anti-inflammatory treatment.[9]

Group 3: Vanadium-treated group received elemental vanadium (1.5 mg/body weight) as sodium metavanadate for 10 consecutive days. This dose of vanadium was dictated by our study, previous, which indicated that the administration of similar amounts of sodium metavanadate resulted in perturbations of brain lipids and lipid peroxidation in discrete rat brain areas.[10]

Group 4: Doxycycline + vanadium-treated group received concomitant dose of vanadium (1.5 mg vanadium/kg + doxycycline [4 mg/kg body weight on day 1 and 2 mg/kg body weight thereafter]) for consecutive 10 days. After 24 hours of the last oral dose, the rats were sacrificed using cervical decapitation. The liver was carefully taken out on an ice-cold surface and stored in deep freeze at −70°C. The relative liver weight of each rat was calculated with a formula as has been given by Adebiyi et al.[11]

Estimation of Lipid Metabolites

The liver tissue (600 mg) was weighed and homogenized in chloroform-methanol (2:1 v/v). The homogenizing medium was dictated by a previous method of Folch et al.[12] The samples were stored at −20°C in a deep freeze for extraction of lipids. The phospholipids were measured spectrophotometrically at 660 nm by phosphate determination.[13] The cholesterol was determined spectrophotometrically at 660 nm.[14] The Cerebroside were measured at 480 nm.[15] The gangliosides were estimated at 580 nm.[16]

Estimation of Lipid Peroxidation, GSH, Ascorbic Acid, Calcium, and Acetylcholinesterase Enzyme

The liver from 30 rats was weighed and divided into equal parts. One part was homogenized in 0.15 M KCl (10% w/v) and was used for the estimation of lipid peroxidation, calcium, GSH, and ascorbic acid levels. Part of the liver was homogenized in 0.2 M ethylenediaminetetraacetic acid (10% w/v) buffer (pH 7.4) and centrifuged at 5000 rpm for 10 minutes. The supernatant obtained was used for the estimation of acetylcholine esterase activity. The content of malondialdehyde (a product of lipid peroxidation) as thiobarbituric acid-reactive substances was spectrophotometrically determined

at 535 nm.[17] The reduced GSH levels were spectrophotometrically determined at 412 nm.[18] The vitamin C levels were spectrophotometrically determined at 535 nm.[19] The calcium was assayed by utilizing Boehringer Mannheim GmbH Diagnosta, Germany (Cat. No. MPR21553593).[20] The activity of AChE enzyme was assayed by utilizing kits supplied by Boehringer Mannheim (Cat no. MPR 21424117) GmbH Diagnosta Germany, and its absorbance was taken at 412 nm.[21]

Statistical Analysis

The data were presented as means ± standard error of ($n = 15$). Data were analyzed by one-way analysis of variance. When the analysis indicated a statistically significant difference ($p < 0.05$), the treated groups were compared with their respective controls. Statistical analyses were performed by F-test[22] for homogeneity of variance followed by Student' *t*-test.

Results

General Observations

The rats administered with physiological saline (control group) or doxycycline alone did not show any sign of illness. However, the rats administered with vanadium displayed signs of neurotoxicity such as akinesia, motoric disturbances, ataxia, convulsion, muscular fasciculation, asphyxia, lethargy, and diarrhea. On the other hand, concomitantly (doxycycline + vanadium)-treated group of rats gained weight and exhibited recovery from illness. These neurological deficits in vanadium neurotoxicity had been previously reported.[23]

Effect of Vanadium on Body Weight of the Rats and Their Mortality

The rats in all the groups were weighed daily throughout the experiment (►**Table 1**). Vanadium-treated group exhibited significant reduction in body weight (−60 g; $p < 0.001$) against controls and (−65 g; $p < 0.001$) against doxycycline-treated group on the day of sacrifice. On the other hand, vanadium + doxycycline-treated group exhibited gain (+25 g; $p < 0.001$) in body weight against vanadium group on the day of sacrifice. Two rats succumbed to death following vanadium administration.

Effect of Vanadium on Relative Liver Weight of the Rats

►**Table 2** shows a statistically significant decrease (−19%; $p < 0.05$) in the relative liver weight of the rats across both saline controls and doxycycline group. On the other hand, vanadium administered group presented statistically significant increase (+28%; $p < 0.001$) in relative liver weight against saline controls and doxycycline group (+58%; $p < 0.001$). Furthermore, doxycycline + vanadium administered group exhibited statistically significant increase (+-35%; $p < 0.001$) across saline controls and doxycycline controls (+65%; $p < 0.001$).

Effects Doxycycline on Levels of Phospholipids, Cholesterol, Cerebrosides, and Gangliosides in the Rat Liver following Vanadium Intoxication

►**Table 3** depicts that vanadium administration significantly depleted lipid profiles as compared with saline controls in the following sequence: cerebrosides (−50%; $p < 0.001$), cholesterol (−39%; $p < 0.001$), phospholipids (−18%; $p < 0.05$). However, levels of gangliosides were significantly elevated (+ 38%; $p < 0.001$), whereas doxycycline + vanadium group significantly protected against the effect of vanadium in the following sequence: cholesterol (+17%; $p < 0.05$), phospholipids (+ 7%; $p < 0.05$), and cerebrosides (+2%). However, doxycycline + vanadium administered group was ineffective in protection against vanadium-induced "Sphingolipidosis."

Table 1 Effect of doxycycline on body weight of rats and their morality following vanadium administration

Group	Treatment	Body weight on day 1 of treatment	Body weight on sacrifice day (g)	Gain or loss in body weight (g)	Mortality
1	Normal saline ($n = 15$)	249 ± 1.42	250 ± 1.93	+ 0.4	0/15
2	Doxycycline (2 mg/kg, oral) ($n = 15$)	250 ± 1.98	255 ± 1.75	+ 2	0/15
3	Vanadium (1.5mg/kg, oral) ($n = 17$)	250 ± 1.65	190 ± 1.16[a,b]	−24	2/17
4	Doxycycline (2 mg/kg, oral) + Vanadium (1.5 mg/kg, oral) ($n = 15$)	247 ± 1.86	215 ± 0.87[a,b,c]	−13	0/15

Abbreviation: SEM, standard error of mean.
n = number of animals in each group. Values are expressed as ± SEM
[a] $p < 0.05$, not significantly different from control.
[b] $p < 0.05$, significantly different from doxycycline.
[c] $p < 0.05$, significantly different from vanadium.

Table 2 Effect of doxycycline on mean values of relative liver weight following vanadium administration

Group	Treatment	Relative liver weight (g) (x 10^{-2})
1	Normal saline ($n = 15$)	3.2 ± 0.27
2	Doxycycline (2 mg/kg, oral) ($n = 15$)	$2.6 \pm 0.25^{a,b}$
3	Vanadium (1.5 mg/kg, oral) ($n = 15$)	$4.1 \pm 0.22^{a,b}$
4	Doxycycline (2 mg/kg, oral) + Vanadium (1.5 mg/kg, oral) ($n = 15$)	$4.3 \pm 0.14^{a,b}$

Abbreviation: SD, standard deviation.
n = number of animals in each group. Values are expressed as ± SD.
[a] $p < 0.05$, significantly different from control.
[b] $p < 0.05$, significantly different from doxycycline.
[c] $p < 0.05$, significantly different from vanadium.

Effect of Doxycycline on Lipid Peroxidation in the Rat Liver following Vanadium Intoxication

►**Table 4** shows that vanadium remarkably accelerated (+64%; $p < 0.001$) the occurrence of lipid peroxidation compared with saline controls. However, doxycycline control group and doxycycline + vanadium administered group exhibited a stringent blockade in occurrence of lipid peroxidation by (−20%; $p < 0.05$) and by (–16%; $p < 0.02$), respectively, as compared to vanadium administered group.

Effects of Doxycycline on Glutathione (GSH) Levels in the Rat Liver following Vanadium Intoxication

The data in ►**Table 4** shows significant reduction in GSH levels (−45%; $p < 0.001$) compared with saline controls following vanadium administration. However, GSH levels were inhibited by (−44%; $p < 0.001$) when compared with both doxycycline-treated group and vanadium + doxycycline-treated group.

Effect of Doxycycline on Vitamin C Levels in the Rat Liver following Vanadium Intoxication

►**Table 4** shows that vanadium administration significantly increased (+ 20%; $p < 0.02$) the levels of vitamin C as compared with saline controls, whereas doxycycline + vanadium-treated group significantly decreased (–14%; $p < 0.05$) the levels against vanadium group.

Effect of Doxycycline on Acetylcholinesterase Activity in the Rat Liver following Vanadium Intoxication

►**Table 4** shows that vanadium administration inhibited (–48%; $p < 0.001$) AChE enzyme against saline controls. However, the synergetic administration of doxycycline vanadium exhibited significant recovery (+ 27%; $p < 0.05$) in the activity of AChE.

Effect of Doxycycline on Calcium Levels in the Rat Liver following Vanadium Intoxication

►**Table 4** shows that vanadium administration resulted in significant increase (+ 80%; $p < 0.001$) in calcium levels but doxycycline alone exhibited decrease (+45; $p < 0.001$) in the calcium levels compared with the saline controls. Moreover, synergistic administration of rats with doxycycline + vanadium had significantly depleted (−17%; $p < 0.05$) the calcium influx in the liver compared with vanadium-treated group.

Discussion

Vanadium-induced lipid peroxidation is toxic to liver cells,[1] brain tissue,[24] and kidney tissue[1] through reactions with cellular lipids, cellular proteins, and nucleic acids therein,[8] thereby, results in cell apoptosis and DNA damage.[1] Many researchers have attempted to counteract the vanadium toxicity with a view to chelate the metal or obviating its oxidant action by the use of chelators such as iron and desferoxamine.[25] We have used antioxidants such as vitamin E, vitamin C, and selenium with varying levels of success in experimental animals.[10,23,26]

In the present study, rats were administered vanadium (1.5 mg/kg body weight). This dose was equivalent to that

Table 3 Effect of doxycycline on the levels of phospholipids, cholesterol, cerebrosides, and gangliosides in the rat liver following vanadium administration

Parameter	Groups			
	Control	Doxycycline	Vanadium	Doxycycline + vanadium
Phospholipids (mg/g fresh tissue weight)	32.74 ± 1.96	32.40 ± 1.10	$26.90 \pm 2.84^{a,b}$	$28.72 \pm 0.59^{a,b}$
Cholesterol (mg/g fresh tissue weight)	2.37 ± 0.09	2.30 ± 0.03	$1.43 \pm 0.08^{a,b}$	$1.67 \pm 0.13^{a,b,c}$
Cerebrosides (mg/g fresh tissue weight)	26.25 ± 1.37	25.75 ± 0.76	$13.1 \pm 1.19^{a,b}$	$13.4 \pm 0.78^{a,b}$
Gangliosides (µg/g fresh tissue weight)	62.55 ± 4.32	62.64 ± 1.90	$86.25 \pm 4.31^{a,b}$	$85.55 \pm 1.82^{a,b}$

Abbreviation: SEM, standard error of mean.
Vanadium (1.5 mg/kg/day), doxycycline (2 mg/kg/day), doxycycline (2 mg/kg/day) + vanadium (1.5 mg/kg/day), and normal saline. All doses were orally administered for 10 consecutive days.
Values are expressed as ± SEM for 15 animals.
[a] $p < 0.05$, significantly different from control group.
[b] $p < 0.05$, significantly different from doxycycline group.
[c] $p < 0.05$, significantly different from vanadium group.

Table 4 Effect of doxycycline on the levels of lipid peroxidation, concentration of reduced glutathione, vitamin C, calcium, and acetylcholinesterase activity in the rat liver following vanadium administration

Parameter	Groups			
	Control	Doxycycline	Vanadium	Doxycycline + vanadium
Lipid peroxidation (nanomole MDA formed/g fresh tissue weight)	129.2 ± 17.06	102.2 ± 25.69[a]	211.6 ± 36.14[a,b]	176.6 ± 27.92[a,b,c]
Reduced glutathione (GSH) (μmole/g/ fresh tissue weight)	0.595 ± 0.022	0.590 ± 0.011	0.330 ± 0.021[a,b]	0.335 ± 0.013[a,b]
Vitamin C (μg/g fresh tissue weight)	396 ± 22.19	391.83 ± 2.40	476 ± 67.58[a,b]	410.55 ± 2.83[a,b,c]
Calcium (μmole/g fresh tissue weight)	0.397 ± 0.12	0.217 ± 0.13 [a]	0.714 ± 0.21[a,b]	0.595 ± 0.13[a,b,c]
Acetylcholinesterase (unit/g fresh tissue weight)	1044.66 ± 136.26	1071.91 ± 114.03	545.04 ± 103.1[a,b]	694.58 ± 196.36[a,b,c]

Abbreviations: MDA, malondialdehyde; SEM, standard error of mean.
Vanadium (1.5 mg/kg/day), doxycycline (2 mg/kg/day), doxycycline (2 mg/kg/day) + vanadium (1.5 mg/kg/day), and normal saline. All doses were orally administered for 12 consecutive days. Values are expressed as ± SEM for 15 animals in each group.
[a] $p < 0.05$, significantly different from control group.
[b] $p < 0.05$, significantly different from doxycycline group.
[c] $p < 0.05$, significantly different from vanadium group.

administered by us[10] in a previous study over a period of 10 days together with a therapeutic dose of doxycycline (2 mg/kg body weight).[9] Vanadium administration to rats displayed neurotoxic effects such as lameness, locomotor deficits, convulsions, and muscular weakness. These neurological disorders may be implicated to production of free radicals. The anorexia and dehydration produced in rats were through diarrhea, leading to the reduction in daily body weight. The increase in relative liver weight following vanadium intoxication might be due to hepatocytes injury as a consequence of the bioaccumulation of vanadium metal. However, coadministration of doxycycline + vanadium presented with no signs of neurological deficits as rats recovered from anorexia their food intake was increased and they gained body weight.

Effect of Doxycycline in Vanadium-Induced Hepatoxicity in Rats on Lipids and Occurrence of Lipid Peroxidation

In this study, hepatoxicity of vanadium presented with significant decreases in phospholipids levels and cholesterol and cerebrosides concentrations. Previous studies have also reported that both, synthesis of cholesterol, and uptake of phosphate into phospholipids in the rat liver were decreased following injection of vanadyl sulfate.[27,28] These reports are well in agreement with our results. Estimation of lipid peroxidation has been proposed as a biomarker for oxidative stress We have estimated enhanced occurrence in lipid peroxidation; therefore, in this study the decrement in liver lipid profiles may also be caused by a reaction of lipids with ROS (lipid peroxidation). This phenomenon has a chain character and leads to oxidative degradation of phospholipids in the cell membranes.[1] The administration of doxycycline with vanadium presented inhibition of lipid peroxidation in rat liver compared with that of vanadium intoxicated group. It is thus likely that doxycycline might have resolved effects of vanadium through antioxidant action. This finding is closely similar to those observed by.[29]

On the other hand, increase in gangliosides concentration was an empirical finding. It may be implicated in human genetic disease referred to as Gaucher's disease. However, doxycycline co-treatment with vanadium protected the integrity of membrane lipids in rat liver, and exhibited an average recovery of phospholipids, cholesterol and cerebrosides by 8.5% against vanadium-treated group. But no protection was presented per se in elevation of ganglioside levels following vanadium toxicity in liver. It crops up a perplexing question, does vanadium affects the activity of β-galactosidase enzyme in hepatocytes and induced "sphingolipidosis"? The results of this study are mirror images of our earlier report, where vanadium neurotoxicity produced exactly similar results in discrete regions of brain.[25] The motif of these studies was to find out subtle intracellular biochemical changes in different organs in response to exogenous substrates.[30] There is still a dearth of literature about this finding and remains to be of high current interest.

Effect of Doxycycline on Glutathione, Vitamin C, Calcium Concentration, and Acetylcholinesterase Activity in Rat Liver following Vanadium Administration

The ability of a cell to maintain functional homeostasis on the induction of protective antioxidant enzymes and intracellular GSH plays a central role in defending cells against oxidative stress.[8] Our results have demonstrated significant reduction in GSH levels following vanadium exposure. On the other hand, co-treatment (vanadium + doxycycline) presented no protection per se against vanadium-induced

inhibition of GSH levels. It is thus likely that the faster rate of occurrence of lipid peroxidation in this study might have resulted in the GSH-linked thiol group participation in protection of cell membranes against oxidative damage; therefore, GSH levels are inhibited.

Detoxification of vanadium by ascorbic acid mainly relies on ascorbic acid-mediated reduction of vanadate to vanadyl and its high capacity to scavenge the ROS.[1] In the present investigation, vanadium treated rats exhibited remarkable increments in the vitamin C levels in the liver. It is thus likely that to ameliorate its hepatoxicity, ascorbic acid might have set an adoptive response to the increased formation of ROS (lipid peroxidation); henceforth, its uptake was increased through blood in a recycling (oxidative reductive) mechanism. The decrease in vitamin C in the (vanadium +doxycycline) group compared with vanadium group might be either due to greater utilization of ascorbic acid in breaking lipid peroxidation reactions or excretion of either vanadium ascorbic acid or doxycycline—ascorbic acid complexes in urine.

Thus far, the in vivo data pertaining to interaction between lipid peroxidation and calcium mediated damage to hepatocytes is ill-defined. However, a singular report by[31] has demonstrated that in the isolated rat liver mitochondria uptake of calcium was stimulated following administration of sodium metavanadate. The results of our study are well in congruence with this report.[32] Furthermore, it is also reported that stimulation of lipid peroxidation increases the intracellular content of calcium in isolated rat hepatocytes[33] therefore, it is suggestive that vanadium triggered Ca^{2+}- dependent increase in lipid peroxidation. The coadministration (doxycycline + vanadium) to rats exhibited a significant decrease in calcium concentration. This is because of the complete prevention of calcium pools in hepatocytes following the antioxidant action of doxycycline. This was curiously parallel with the inhibition of lipid peroxidation against vanadium group. It is suggestive that the doxycycline, being an antioxidant and chelating agent, therefore, it administered completely prevented high Ca^{2+} influx into hepatocytes by inhibition of lipid peroxide formation and chelation of vanadium to diminish its toxicity.

AChE enzyme is rich in liver and it is as a biomarker of vanadium toxicity.[25] In this study, its activity was significantly inhibited in vanadium group. It is speculative that it was caused by the perturbations in biophysical properties in hepatocyte cell membrane following lipid peroxidative damage. On the other hand, co-treatment (doxycycline+ vanadium) treatment exhibited reversal (increase) in the activity of AChE. This may be a consequence of antioxidant action of doxycycline via inhibition of lipid peroxidation. There is a dearth of information regarding this study and further research is warranted in future.

Conclusion

In summary, this study supported the hypothesis that vanadium-induced oxidant stress and depletion of cellular lipids and antioxidants play a key role in sharp rise in injury to hepatocytes membrane (cytotoxicity). Furthermore, this study unraveled the protective role of doxycycline with special reference to lipid metabolites, cellular GSH, vitamin C, calcium, and AChE enzyme against vanadium in rat hepatocytes. The enormous release of vanadium into environment is increasing and assessment of its effects on human health should be taken into consideration; therefore, further studies are in progress to lend a better insight into the therapeutic role of doxycycline in ameliorating deleterious effects of vanadium.

Limitation of This Study

While we were working in this project, our purchasing agents had difficulty in procuring diagnostic kits for antioxidant enzymes.

Conflict of Interest

None declared.

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