Lactational Vitamin E Protects Against the Histotoxic Effects of Systemically Administered Vanadium in Neonatal Rats

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Summary: The work investigated the protective role of lactational vitamin E administration on vanadium-induced histotoxicity. Three groups of Wistar rats, with each group comprising of two dams and their pups, were used in this study. Group I pups were administered intraperitoneal injection of sterile water at volumes corresponding to the dose rate of the vanadium (sodium metavanadate) treated group from postnatal day (PND) 1-14 while those in Group II were administered intraperitoneal injection of 3mg/kg vanadium from PND 1-14. Group III pups were administered intraperitoneal injection of 3mg/kg vanadium while the dam received oral vitamin E (500mg) concurrently every 72hours. The results showed that group II pups exhibited histopathological changes which included seminiferous tubule disruption of the testes characterised by vacuolar degeneration and coagulative necrosis of spermatogonia and Sertoli cells with reduction in mitosis, and areas of interstitial thickening with fibroblast proliferation. In addition, the lungs showed disruption of the bronchiolar wall and denudation of the bronchiolar respiratory epithelium while the liver showed hydropic degeneration and coagulative necrosis of the centrilobular hepatocytes. These histotoxic changes were ameliorated in the vanadium + vitamin E group. We conclude that lactational vitamin E protects against the histotoxic effects of vanadium and could be a consideration for supplementation in the occupationally and environmentally exposed neonates. However, caution should be taken in vitamin E supplementation because there is still equivocal evidence surrounding its benefits as a supplement at the moment.

**Keywords*:* Vanadium, Vitamin E, Histotoxicity, Antioxidant.

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INTRODUCTION

Vanadium is a major trace metal in fossil fuels that is used extensively in modern industries in the manufacture of jet air craft, phthalic anhydride, pesticides, sulphuric acid, photography and as a catalyst in the production of many materials (Wenning and Kirsch, 1988). Acute environmental and occupational exposure to vanadium and its compounds is however not uncommon (Hope, 1994) since it is a transition metal widely distributed in nature and extensively used in heavy industry (Ray et al., 2006). Sources of occupational exposure include combustion of vanadium-rich fuels, processing and refining of vanadium ores and sludges, manufacturing of vanadium-containing products, and by handling of catalysts in the chemical industry (Plunkett, 1987). With inhalation being the major route of environmental exposure (Barceloux, 1999), combustion of fossil fuels provides a significant environmental source of vanadium. The increase in environmental levels of vanadium has raised concern over the release of the element into the atmosphere from anthropogenic sources (Hope, 1994), and recently from massive oil burning as seen in Arabian Gulf (Haider *et al*, 1998), the Niger-Delta region of Nigeria (Igado *et al*., 2008), and the Gulf of Mexico

(Olopade and Connor, 2011). The general population is also increasingly exposed to this metal mostly as a result of the increased utilization of vanadium containing petroleum fuel (Byczkowski and Kulkarni, 1996).

A variety of toxic effects are exerted by vanadium compounds and these effects are dependent on the oxidation state and circulating levels of vanadium. However Sanchez *et al.* in 1998 and Soares *et al.* in 2008 showed that V^{5+}(oxidative state) seems to be more toxic than V^{4+} and V^{3+}. The most affected organs, as documented by histopathological alterations, were the liver and kidney (Valko *et al.,* 2005) and intraperitoneal injections of rats with orthovanadate induced nephrotoxicity (Ciranni *et al,* 1995). The reproductive and developmental functions of rats have also been well established to be affected by vanadium (Morgan, 2003).

Vanadium, with an atomic number of 23 on the periodic table; is a transition metal element with a complex chemistry as it forms polymers frequently and can occur in various oxidative states (Nechay, 1984). Vanadium participates in reactions involving formation of reactive oxygen species and free radicals (Crans *et al,* 2004) so it is therefore not surprising that antioxidants offer protection from vanadium toxicity (Olopade et al., 2011a).

Vitamin E, a member of the fat-soluble vitamins, prevents oxidative stress by working together with a group of nutrients that prevent oxygen molecules from becoming too reactive. This group of nutrients includes vitamin C, glutathione, selenium, and vitamin B3 (George, 2010). It scavenges peroxyl radicals and binds with them to form a tocopheryl radical which can then be reduced by a hydrogen donor to its reduced state (Traber *et al,* 2011). Because vitamin E is fat soluble, it is incorporated into cell membranes and protects them from oxidative damage.

This work focused on the protective role of vitamin E on the vanadium induced histopathological changes in the liver, lungs, and testes of neonatal Wistar rats.

MATERIALS AND METHODS

Animals

Six adult female Wistar rats were housed in the experimental animal house of the Neuroscience Unit of the Department of Veterinary Anatomy, Faculty of Veterinary Medicine, University of Ibadan, Ibadan, Nigeria. They were acclimatised for a period of two weeks after which they were bred. The animals were fed with pelleted rat feed with water supplied *ad libitum*. The University of Ibadan Ethical Guideline on Use of Animals in Research was followed in this study.

Study design

There were three groups, each comprising2 dams and their pups. For each group, 6 pups were randomly selected (n=6)

Group I: Pups injected with sterile water once daily intraperitoneally (IP) from postnatal day 1-14 (PND1-14).

Group II: Pups were administered sodium metavanadate, 3mg/kg body weight once daily IP from PND 1-14 based on the work of Todorich*et al* 2011.

Group III: Pups were administered sodium metavanadate 3mg/kg body weight IP from PND 1-14; the dams (n=2) were concurrently administered oral vitamin E (500mg) every 72 hours to deliver it to the pups via lactation (Martin and Hurley, 1977).

Preparation of tissues

The pups were anaesthetised with ketamine (100mg/kg) IP on PND 15. They were then quickly decapitated and dissected on ice. The lungs, liver, and testes were harvested. The testes were immediately fixed in Bouin's fluid while the lungs and liver were fixed in 10% formalin for 72hours.

Histopathology of tissues

Samples of harvested lungs, testes and liver were thoroughly dehydrated by passing them through graded solutions of alcohol (30%, 50%, 70% and 100%) after which they were cleared in two changes of xylene at an interval of one hour each and

impregnated with three changes of paraffin wax for an hour each and finally embedded in fresh molten wax. Sections of 5µm thickness of each organ were prepared and stained with haematoxylin and eosin for examination under a light microscope.

RESULTS

Histopathology of the testes

In group II (vanadium only group), there was disruption of seminiferous tubules characterised by vacuolar degeneration and coagulative necrosis of spermatogonia and Sertoli cells with reduction in mitosis, and areas of interstitial thickening with fibroblast proliferation. These were absent in the vanadium+vitamin E and control groups (Fig.1)
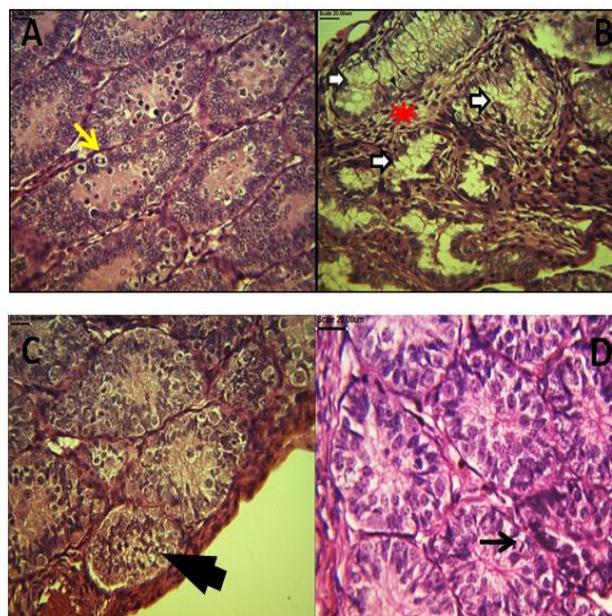

Figure 1: Transverse section of the seminiferous tubules (H&E, x400): A- control: yellow arrow showing a dividing cell; B and C: vanadium only; white arrows pointing to seminiferous tubules with severe loss of the germinal epithelium due to vacuolar degeneration and necrosis, red star indicating area of interstitial thickening; black arrow pointing to a tubule with necrotic germinal cells; D: vanadium + vitamin E; black arrow showing a dividing cell.

Histopathology of the lungs

There was disruption of the bronchiolar wall and denudation of the bronchiolar respiratory epithelium in the lungs of the vanadium only group; this was in contrast with the well-defined smooth muscle layer of the bronchiolar walls of the vanadium+ vitamin E and control groups. (Fig.2).

Histopathology of the liver

There was hydropic degeneration and coagulative necrosis of the centrilobular hepatocytes in the vanadium only group. This was absent in the vanadium + vitamin E and control groups. (Fig.3)
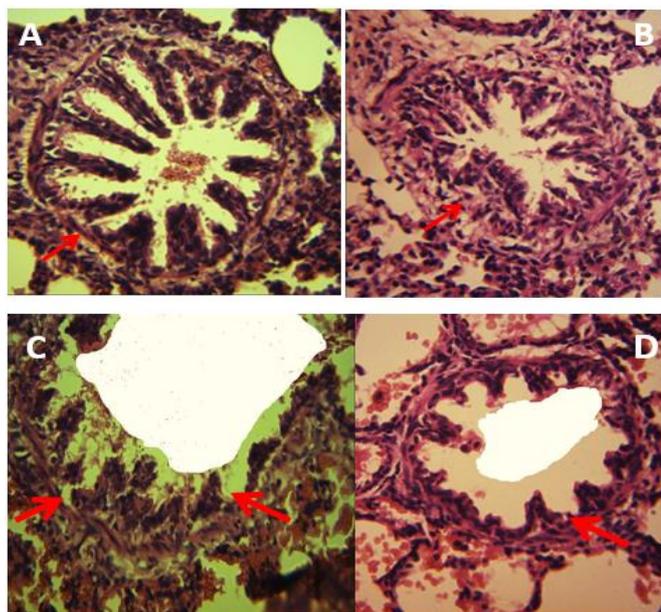
Figure 2: Bronchioles, Cross Section, H&E x400: A-control; the bronchiolar architecture is well defined with the arrow pointing to the smooth muscle of the laminar propria. B-vanadium only; there is disruption of the bronchiolar smooth muscle/connective tissue (red arrow). C-vanadium only;the red arrows are pointing to areas of epithelia sloughing.D-vanadium+ vitamin E; note the intact smooth muscle/connective tissue layer in the lamina propria (red arrow)
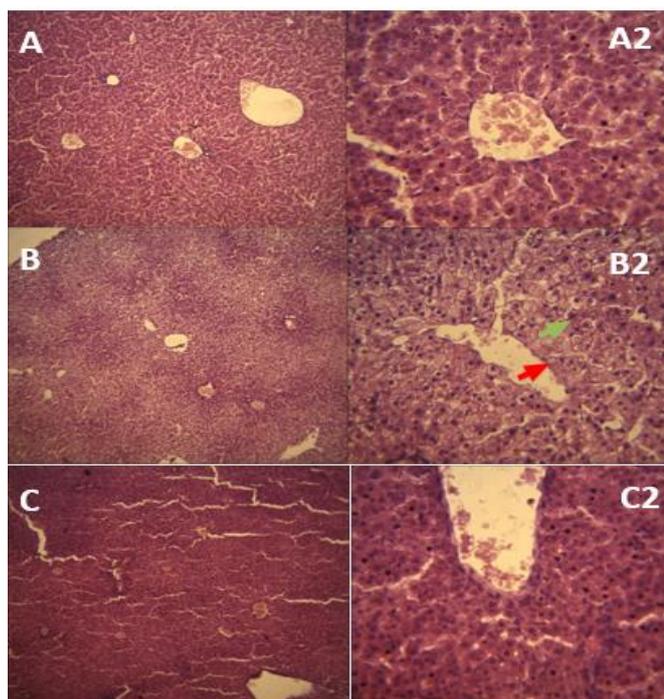

Figure 3: Liver, Transverse Section, H&EA: Control showing the normal liver architecture (x100) A2: Control, centrilobular venule (x400) B: Vanadium only. The pale areas around the central veins are areas of hydropic degeneration and coagulative necrosis (x100) B2-Vanadium only(x400).Hepatocytes of the centrilobular zone are necrotic (red arrow) with others showing hydropic degeneration (green arrow) C: Vanadium + vitamin E (x100) normal liver architecture. C2- Vanadium + vitamin E (x400) showing no visible pathology.

DISCUSSION

Vanadium induces production of reactive oxygen species which have a range of deleterious effects on the cell metabolism such as lipid peroxidation, denaturation of intracellular proteins and destruction of DNA (Shi *et al,* 1996). In this study, vanadium was shown to induce necrotic and degenerative changes in the testes, lungs and liver.

In the testes, vanadium inducednecrosis and vacuolar degeneration of the spermatogonia and Sertoli cells coupled with reduction in mitosis. Vanadium has been reported to induce G2/M phase arrest in a time- and dose-dependent manner (Zhang *et al,* 2001); this most likely explains the reduction in mitosis in the vanadium exposed testis.

In the liver, vanadium exposure resulted in marked centrilobular hepatocellular injury, characterised by hydropic degeneration and coagulation necrosis. Hepatocytes in the centrilobular zone have a high composition of cytochrome p-450 enzymes (Stalker and Hayes*,* 2007). These enzymes have been implicated in the production of reactive oxygen species (Halliwell and Gutteridge, 1990). Thus, the pronounced centrilobular necrosis and degeneration can be said to be expected in the vanadium exposed group.

A previous study on vanadium pentoxide showed that it induces bronchial hyper responsiveness and asthma in vanadium plant workers (Irsigler *et al*, 1999). In this current study on sodium metavanadate, the disruption of the bronchiolar smooth muscle observed might explain the asthma-like symptoms seen in people exposed to vanadium via inhalation.

In 2011, Traber *et al.* established that vitamin E binds with peroxyl radicals to form a tocopheryl radical which can then be reduced by a hydrogen donor and this antioxidative property of vitamin E is effective within a cell as vitamin E is fat soluble is easily incorporated into cell membranes. Vitamin E administration attenuated the histotoxic effects of vanadium, as shown above, on the testes, liver and lungs of the pups. This result agrees with both the earlier report by Olopade *et al* (2011b) who administered both vanadium and vitamin E via the lactation route and with the work of Uche *et al* (2008) who showed that vitamin E prevented and reversed morphophysiological deficits induced by vanadium in the testes and liver.Vitamin E via the lactation route has also been shown to protect neonatal rats from vanadium-induced reactive astrogliosis and behavioural deficits (Olopade *et al.,* 2011a).

It can be deduced from this study that lactational vitamin E administration protects against the histotoxic effects of vanadium and could be considered for supplementation in the occupationally and environmentally exposed neonates. However, caution should be taken in vitamin E supplementation because there is still equivocal evidence supporting

its benefits as a supplement at the moment (Haber, 2006; Gallo *et al*., 2010; Abner *et al,* 2011; Olopade *et al.,* 2011b)

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