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Neurobehavioral and cytotoxic effects of vanadium during oligodendrocyte maturation: A protective role for erythropoietin

Oluwaseun Mustapha[a,b], *Bankole Oke*[a], *Nils Offen*[c,1], *Anna-leena Sirén*[c], *James Olopade*[a,c,*]

[a] *Department of Veterinary Anatomy, University of Ibadan, Nigeria*
[b] *Department of Veterinary Anatomy, Federal University of Agriculture, Abeokuta, Nigeria*
[c] *Department of Neurosurgery, University of Würzburg, Josef-Schneider Strasse 11, 97080 Würzburg, Germany*

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ABSTRACT

Vanadium exposure has been known to lead to lipid peroxidation, demyelination and oligodendrocytes depletion. We investigated behaviour and glial reactions in juvenile mice after early neonatal exposure to vanadium, and examined the direct effects of vanadium in oligodendrocyte progenitor cultures from embryonic mice. Neonatal pups exposed to vanadium *via* lactation for 15 and 22 days all had lower body weights. Behavioural tests showed in most instances a reduction in locomotor activity and negative geotaxis. Brain analyses revealed astrocytic activation and demyelination in the vanadium exposed groups compared to the controls. In cell culture, exposure of oligodendrocytes to 300 μM sodium metavanadate significantly increased cell death. Expression of the oligodendrocyte specific proteins, 2′,3′-cyclic nucleotide 3′-phosphodiesterase (CNPase) and oligodendrocyte specific protein (OSP/Claudin) were reduced upon vanadium treatment while simultaneous administration of erythropoietin (EPO; 4–12 U/ml) counteracted vanadium-toxicity. The data suggest that oligodendrocyte damage may explain the increased vulnerability of the juvenile brain to vanadium and support a potential for erythropoietin as a protective agent against vanadium-toxicity during perinatal brain development and maturation.

1. Introduction

The world atmospheric vanadium concentration has been on the rise (Hope, 1994) and increased accumulation of vanadium has been reported in tissue samples of humans in high exposure areas over decades (Fortoul et al., 2002). Vanadium is released naturally into the atmosphere through marine aerosols, forest fires, volcanic emissions and formation of continental dust (Englert, 2004). In addition, vanadium is a constituent of virtually all coal and petroleum oils (Eckardt, 1971). Consequently, the recent increases in incidental and

* *Corresponding author at*: Comparative Anatomy, Environmental Toxicology and Neuroscience Unit, University of Ibadan, Ibadan, Nigeria. Tel.: +234 8023860829.
E-mail addresses: drmustyplato@yahoo.co.uk (O. Mustapha), bankoleoke@yahoo.com (B. Oke), nils.offen@med.lu.se (N. Offen), siren.a@ukw.de (A.-l. Sirén), jkayodeolopade@yahoo.com (J. Olopade).
[1] Present address: Lund Stem Cell Center, BMC B10 Lund University, Klinikgatan 26, 221 84 Lund, Sweden.
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accidental burning of crude oil as seen in the Arabian Gulf, the Nigerian Niger Delta and the Gulf of Mexico have been estimated to be sources of additional particulate vanadium in the atmosphere (Olopade and Connor, 2011; Scheren et al., 2002).

Inhalation of vanadium is known to lead neuropathological lesions (Avila-Costa et al., 2004). Intraperitoneal injections of vanadium cause lipid peroxidation and demyelination (Garcia et al., 2005, 2004) in adult rats and lead to hypomyelination and damage to the myelin producing oligodendrocytes in neonatal rats (Todorich et al., 2011). Placenta is only a partial barrier against vanadium (Edel and Sabbioni, 1989) and vanadium is present in breast milk of exposed mothers (Barceloux, 1999). Since vanadium easily passes the blood brain barrier (Barceloux, 1999) it could cause developmental neurotoxicity in the exposed foetal brain. In fact, developmental delays and deficits in myelination have been reported in neonatal rats exposed to vanadium through their mother's milk (Olopade et al., 2011; Soazo and Garcia, 2007).

Erythropoietin (EPO) is a primary stimulator of red cell production through promotion of survival, proliferation and differentiation of erythroid progenitor cells (Sirén et al., 2009). The neuroprotective properties of EPO have been the topic of intensive study over the last decade (Sirén et al., 2009; Aschner et al., 2010). During a hypoxic event in the brain, Hypoxia Induction Factor (HIF)-1a stabilization is promoted, which in turn activates hypoxia-sensitive genes (Aschner et al., 2010) inducing transcription of endogenous EPO from Neurons and Astrocytes (Ponce et al., 2013). Endogenous and exogenous EPO are capable of binding the extracellular EPO receptor (EPOR), causing its homodimerization. Once EPO has coupled with EPOR, several downstream signalling pathways including phosphatidylinositol-3-kinase (PI3K) are activated and this through different intermediaries subsequently leads to inhibition of caspase formation. Additionally, EPO has been shown to enhance the antioxidant defense mechanisms, increases nitric oxide (NO) production, resulting in cerebral vasodilation and induces the transcription of anti-apoptotic genes (Ponce et al., 2013).

The present study investigated behaviour and brain histology of juvenile mice during early postnatal age (onset and peak of myelination) through exposure of lactating mothers to vanadium. Additionally, the cellular effects of vanadium toxicity and the protective role of EPO on vanadium induced cellular changes were studied in developing oligodendrocyte precursor cells.

2. Materials and methods

2.1. Animal experiments

All experiments were approved and carried out in accordance to guidelines of the animal use and ethics committee of the University of Ibadan.

2.2. Experimental design

Nursing CD-1 mice with their pups were used for this experiment. Both male and female mice were bred and housed in the experimental animal house of the neuroscience unit of the Department of Veterinary Anatomy, University of Ibadan. The animals were pellet fed, given tap water *ad libitum* and were kept at 27 °C with 12 h each of light and dark cycles. The animals were divided into four groups (A, B, C, D) with two nursing dams and their respective pups forming a group. Each dam had an average of 6 pups across all groups, thus making 12 pups per group. Animals were kept as one pregnant dam with her pups in one cage.

Group A (acute vanadium group, V15): sodium metavanadate (3 mg/kg body weight SMV, Sigma-Aldrich, St. Louis, USA) was administered to nursing dams intraperitoneally (IP) for 14 days from postnatal day (PND) 1 and the mice were sacrificed on the 15th day.

Group B (acute control group, C15): sterile water was administered IP to nursing dams for 14 days from PND1 and the mice were sacrificed on the 15th day.

Group C (chronic vanadium group, V22): SMV for 21 days IP to nursing dams from PND1 and the mice were sacrificed on the 22nd day.

Group D (chronic control group, C22): sterile water was administered IP for 21 days and the mice were sacrificed on the 22nd day.

The mice of groups A, B and groups C, D were sacrificed on PND 15 and 22, respectively prior to which behavioural tests were carried out.

2.3. Sample collection

The mice were anaesthetized with ketamine and then perfused transcardially with 4% phosphate buffer formalin with the aid of a perfusion pressure pump and brains were removed according to the method described by Olopade et al. (2011). Briefly, the frontal, parietal and temporal bones were removed to expose the brain which was gently scooped out.

2.4. Behavioural tests

The mice pups were tested on the open field, and for negative geotaxis as described by Garcia et al. (2004) and Soazo and Garcia (2007).

Open field test: Each mouse was placed in the centre of a square cage (120 cm × 120 cm). The floor was divided into 20 cm^2 drawn in black ink. The mice were allowed to move freely around the open field and explore the environment for 5 min. It provides simultaneous measures of locomotion, exploration and anxiety. The following behaviours were scored according to Brown et al. (1999):

1. *Line crossing*: Frequency with which the mice crossed one of the grid lines with all four paws.
2. *Centre square entries*: Frequency with which the mice crossed one of the red lines with all four paws into the central square.
3. *Centre square duration*: Duration of time the mice spent in the central square.
4. *Rearing*: Frequency with which the mice stood on their hind legs in the maze.