

Dr. Idris A. AZEEZ

REPORT ON RESEARCH VISIT– CATEGORY 1A

Home Institution: Neuroscience Unit, Department of Veterinary Anatomy, University of Ibadan, Nigeria.

Host Name: Prof. Marina Bentivoglio

Host Institution: Section of Anatomy and Histology, Department of Neurological and Movement Sciences, School of Medicine, University of Verona, Italy.

Project title: Characterization of the neuroinflammatory response to chronic vanadium-induced neurotoxicity.

Visit: 12 November 2013 – 9 February 2014

Introduction

The project implied to process in the host laboratory brains from mice exposed to vanadium, in order to receive training in the processing of brain sections and quantitative data analyses. Behavioural testing was done in the home institution, as agreed, prior to departure.

I arrived at the University of Verona on the 12th November 2013 and was introduced to the management, staff and students working in my host laboratory. Dr. Anna Andrioli (my tutor) showed me the equipment, laboratories, office space and all the necessary support towards a smooth settling, learning and research.

All reagents (chemicals and antibodies) used for the project during my stay were provided by my host, who also provided lodging in a guest room of the Medical School for the first two months (I stayed then in a students' apartment)

Experimental vanadium intoxication, body weight monitoring and behavioural testing

(Home Laboratory):

Prior to my departure for Verona, animal experiments were conducted, under ethical permission, at the University of Ibadan, using a previously standardized mouse model of vanadium intoxication (Olopade et al., 2011).

Briefly, the experimental design implied vanadium exposure of suckling mice via treatment of lactating dams (vanadium is known to be secreted in the milk of exposed dams; Olopade et al., 2011), and of the mice after weaning.

Mice were randomly assigned to vehicle or vanadium treatment. The dams were administered sodium metavanadate (NaVO_3 ; 3 mg/kg body weight, ip) throughout the lactation period, i.e. from postnatal day (P) 1 through P21 of the pups, which were then treated ip with calculated dose (based on body weight changes) of NaVO_3 3 times a week until 3 months of age (i.e. from P22 through P89). Matched controls were treated ip with sterile water with the same regimen. Body weight was monitored during the first 3 weeks (P1-P22) and then twice weekly until P86

The animals were subjected to behavioural testing at P60 and P89 for hanging wire test and at P89 for the open field tests. At the time of sacrifice (P90), the animals were anesthetized with Ketamine (100 mg/kg, ip) and then perfused transcardially with 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4. The brains were postfixed for 2 h in the fixative solution and then transferred to 0.1% sodium azide in 0.01M phosphate-buffered saline (PBS). The brains were then transferred to the host laboratory.

Brain processing (*Host Laboratory*)

Fixed brains were soaked in 30% sucrose in PBS for cryoprotection for 24 h prior to cutting on a freezing microtome. Serial frozen sections were then cut through the brain in the coronal plane at a 30 μm thickness, and processed with the techniques summarized below.

Black gold histochemistry for myelin

Brain sections were processed for myelin staining using the Black Gold histochemical protocol by Schmued et al. (1999), as modified by Savaskan et al (2009).

Immunohistochemical studies of microglia, astrocytes and pro-inflammatory cytokine expression

Sections were processed for immunoperoxidase according to the protocol used by Andrioli et al. (2009). Series were randomly chosen for immunolabelling of microglia (using rat anti-CD11b

primary antibodies) and interleukin (IL)-1 β (using goat anti-IL-1 β primary antibodies). This material was examined in bright-field microscopy.

Astrocytes were investigated using glial fibrillary acidic protein (GFAP) as a marker, which was revealed, together with tumor necrosis factor (TNF)- α expression, by double immunofluorescence. Sections were then counterstained with the fluorescent nuclear marker DAPI. This material was investigated in confocal microscopy

Quantitative analyses

The sections processed with immunoperoxidase were analyzed (densitometric analysis of immunosignal intensity and extent of the area covered by microglial cells in the neocortex comparing control and vanadium-treated brains), using Image Pro-Plus 7.0

Results and overall outcome of the award

Highlights of results obtained are as follows:

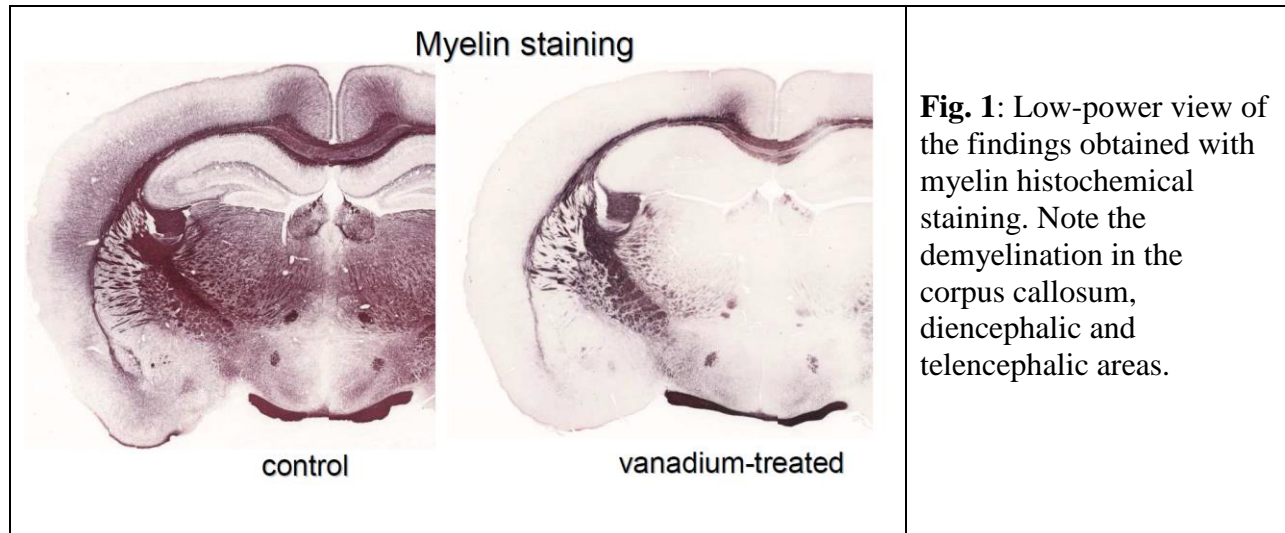
Animal testing

1. A significant reduction of body weight gain was documented in vanadium-exposed mice compared to controls. As for the temporal progression of this deficit, no significant body weight variation was found in the vanadium-treated mice during the first two weeks (P1-14). In the study, body weight reduction was found from the third postnatal week through the third month.
2. Reduction of locomotor and exploratory activity after long-term vanadium exposure: the open field test, performed at P89, showed a significant reduction in all parameters except for rearing, indicating impairment in the animals' basal locomotor activity and exploration. The hanging wire test showed a tendency towards a latency decrease at P60 and P89, which, however, did not reach significance.

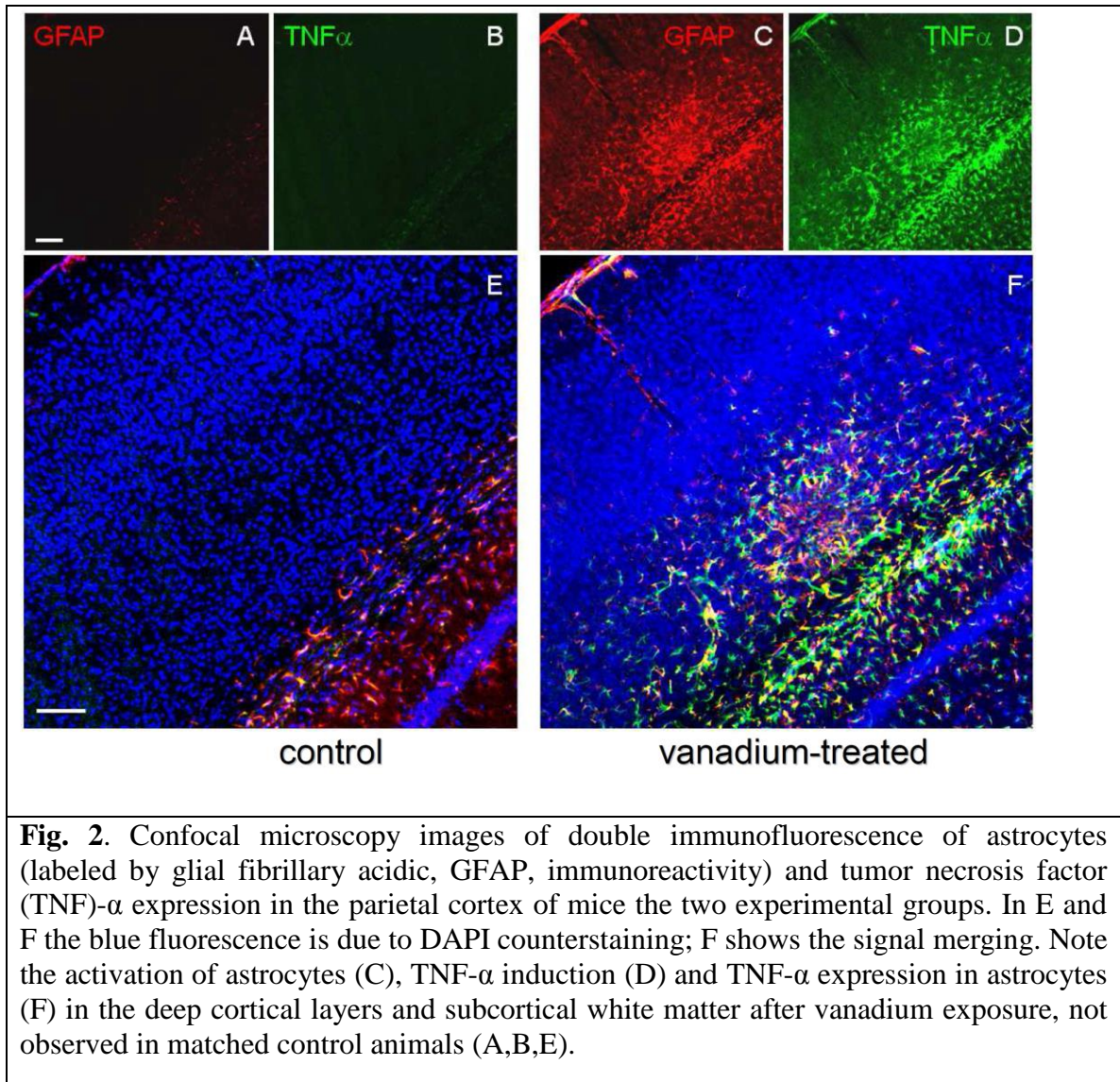
Brain processing

3. Marked demyelination was observed in the vanadium-treated mice compared to control (**Fig. 1**). Findings showed myelin damage especially in commissural fibers and in gray

matter regions on the neocortex, hippocampus and diencephalon, after exposure to vanadium in the first three months of life.



4. The study revealed features of microglial cell activation (cell body hypertrophy and thickening of processes) in several brain regions (including the neocortex, the dentate gyrus, hippocampal CA1 and CA3 fields, thalamus and lateral and posterior hypothalamic areas) after vanadium exposure.
5. Astrocytic activation was also observed, and was especially marked in the corpus callosum and other fiber tracts such as the internal capsule, the deep layers of the neocortex, hippocampal fields, and the habenular complex.
6. Concerning the expression of the pro-inflammatory cytokines, induction of IL-1 β was observed in diencephalic regions after vanadium exposure, but the findings require confirmation and further control of the antibody specificity. Striking results were instead observed on induction of TNF- α expression in the brain of the vanadium-treated mice, in which the cytokine was found to be induced in astrocytes (as shown by double immunofluorescence) (**Fig. 2**), including TNF- α induction in astrocyte endfeet apposed to cerebral microvessel walls.



Benefits of the award

In my three month-stay at the University of Verona, I was able to learn *i*) the use of freezing sliding microtome in brain sectioning; *ii*) routine histological (Nissl) staining; *iii*) principles of histochemistry and immunohistochemical techniques; *iv*) data collection (unbiased cell counts) at the microstereoinvestigator and analysis using Image Pro-Plus 7.0; *v*) image acquisitions at the confocal microscope.

With ISN duly acknowledged, findings from my stay in Verona were presented by Prof. James Olopade at the Forum Federation of European Neuroscience Society (FENS) 2014 (*J.*

Olopade, I.A. Azeez, C. Laperchia, A. Andrioli, M. Bentivoglio; Poster no: C137; Poster Session - Neurotoxicity, inflammation, and neuroprotection, Poster boards: C099-137). The manuscript is now at an advanced stage of preparation.

Work done in Verona enabled me complete and defend my Masters of Science (M.Sc.) dissertation, under the supervision of Prof. James Olopade at the University of Ibadan, Nigeria on August 16th, 2014.

Also, my exposure during the short stay gave me the opportunity to know about the University of Verona Graduate programs. I applied to the PhD Program in Neuroscience and I passed the admission exam in October 2014.

Currently, I am continuing my training and education as a PhD student with scholarship, under the supervision of Prof. Bentivoglio in the Neuroscience Program of the University of Verona. I am committed to return to my home institution after my PhD Thesis defense.

Words cannot express my gratitude to ISN-CAEN, my Professors, James Olopade and Marina Bentivoglio, and tutors, Drs. Funmi Olopade, Anna Andrioli and Claudia Laperchia.

Thanks a lot for the ISN-CAEN support!

References cited

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Schmued L., Slikker W. J. (1999) Black-Gold: a simple, high-resolution histochemical label for normal and pathological myelin in brain tissue sections. *Brain Res.* **837**, 289–297.



Picture taken in January, 2014 at one of the microscope rooms in the host laboratory (Section of Anatomy and Histology, Department of Neurological and Movement Sciences, School of Medicine, University of Verona, Italy).