Neurotoxic aspects of tungusten Alloy Based Heavy Metals in Rat Brain: A DTI study

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Target audience: Researchers and students.

Purpose: Heavy metal–tungsten alloys (HMTAs) are dense heavy metals composed of a mixture of tungsten (W) (91–93%), nickel (Ni) (3–5%) and either cobalt (Co) (2–4%) or iron (Fe) (2–4%) particles. HMTAs is increasingly adopted as the raw material to make parts of military products, such as bullet, armor and shells, shrapnel head, grenade, hunting gun, etc. They have been introduced in an attempt to find safer alternatives to depleted uranium and lead munitions. However, all the three metals used in HMTAs are known to cross the blood brain barrier. However, even the very low dose of metals reaching to the brain may be harmful without the damage being observed. Therefore internalization and retention of these metals in brain may leads to neurotoxicity and may also leads neurophysiological disorders at later stage. Magnetic resonance imaging (MRI) has proven to be very sensitive and excellent technique for detecting the anatomical and pathological changes occurring in brain. Identifying an early sign of normal tissue damage with MRI non invasively, would have the potential to predict organ dysfunction/injury prior to its clinical manifestation. However, among the various MRI sequences used, diffusion tensor imaging (DTI) allows early in vivo examination of tissue structure to be probed and imaged in a microscopic scale, providing clue to the fine architecture of the neural tissue and to changes associated with various physiological and pathological changes[1].

Aim: The aim of our study was to investigate the role of quantitative DTI in defining the microstructural damage in rat brain after acute exposure to the metals used in HMTAs as well as their combined effect by using DTI technique.

Materials and methods: Male Sprague Dawley rats of 11 weeks of age and weighing 250-300g (n = 30) were taken and acclimatized for 48 hours in polypropylene cages under standard temperature, humidity conditions prior to group allocation and treatment. All the 30 rats were divided into 5 groups; control group and treatment groups for each of the respective individual metal salts $NiCl_2$, $CoCl_2$, $Na_2WO_4.2H_2O$ and their mixture (mixed in proportion of military relevant mixture consisting of 91% W: 5% Ni: 4% Co) prepared in 0.9% saline. Animals (n=6 in each treated group) were injected intraperitonealy (i.p.) with individual metal salts and their mixture. Controls (n=6) were injected with 0.9% saline (i.p.). Brain MRI experiments were performed in controls and at day 1, day 3 and day 5 post dose by first anesthetizing the animals with the cocktail of ketamine and xylazine. All MR imaging was performed in a Bruker Biospin 7.0 Tesla 30 cm horizontal bore magnet (Bruker Biospin Ettlingen, Germany) with resonant frequency of 300 MHz. Signal excitation was accomplished with a 72-mm inner diameter (ID) linear birdcage coil, and signal reception was achieved using phase array coil for rat head. MRI protocol included high-resolution anatomical RARE images and DTI images. DTI images were acquired using a multi-slice, multiple-shot spin echo EPI sequence with the following parameters: repetition time (TR) / echo time (TE) = 3800 ms/31 ms, number of gradient encoding directions = 46, and b= 672 s mm-2. The other parameters were: acquisition matrix = 128×128, field-of-view = 4 cm × 4 cm, slice thickness =1 mm and number of slices = 15 (contiguous). Java based DTI analysis software was used for the generation of FA (Fractional Anisotropy) and MD (Mean Diffusivity) maps[2]. Regions of interest (ROI)s were placed on corpus callosum (CC), cingulum (CG), sensory-motor cortex (SMC), cudato-putamen (CUP), thalamus, hypothalamus, hippocampus (Hip) and cerebral peduncle (CP) (Figure 1). FA and MD values from right and left hemisphere were pooled together for statistical analysis. Multivariate analysis of variance (MANOVA) with multiple comparisons using Bonferroni, Post Hoc test was performed to evaluate the differences in DTI measures among different time points.

Results and Discussion: At any time points, no abnormalities were observed in any group on anatomical images. Not much change was observed in the FA values. A significantly increased MD values were observed at day 5 in thallamus in animals treated with of all the three individual metal salts as well their mixture with respect to the control group (Figure 2). Apart form this white matter region of the brain mainly the CP also showed an increase in MD values only in case of individual metal salt. The MD values in CP were found to be increased with respect to control in case of tungsten at day 1 and at day 3 and day 5 in case of cobalt (Figure 3).

In our study we have observed that all the three metal salts have an effect on both the gray and white matter regions of the brain. In addition to this when present together in military relevant proportion they have pronounced effect on the thallamic region of the brain.

The brain MD changes are a function of intracellular-extracellular water homeostatis[3]. The heavy metals used in HMTAs also replace or mimic Ca^{2+} and other ions in many of the important physiological processes of the body. They might have caused alteration in Ca^{2+}-ATPase pump activity which might have caused change in cell membrane permeability. The increase in MD values in our study might be due to increased interstitial space, which might be due to reduced neural or glial cell packing or cell size, or decreased water exchange rate between the intra- and extracellular compartments[4]. Our results demonstrate acute exposure of heavy metals used in HMTAs induced microstructural changes in brain parenchyma and have an effects both the gray and white matter region of the brain which can be detected in early acute phase even before conventional MRI.

Conclusion: The present finding suggest that the heavy metal used in HMTA's are neurotoxic and DTI may be a promising tool for detecting and monitoring the early metal induced changes in brain non-invasively.

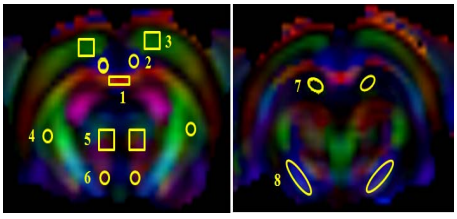

Figure 1. Color-coded FA map from a male age matched rat shows region of interest placement on corpus callosum (CC) (1), cingulum (CG) (2), sensory-motor cortex (SMC) (3), cudato-putamen (CUP) (4), thalamus (5), hypothalamus (6), hippocampus (Hip) (7) and cerebral peduncle (CP) (8).

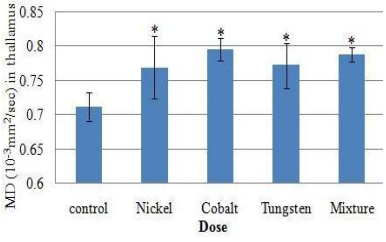

Figure 2. Bar graph showing change in MD values in thalamus region of the brain at day 5 in all the three individual metal salts post dose groups and their mixture with respect to control. * denotes significant difference with controls ($p<0.05$).

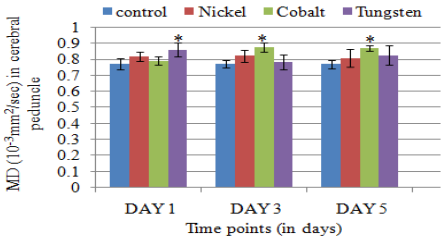

Figure 3. Bar graph showing change in MD values in cerebral peduncle region of the brain at different time points in control and all three individual metal salts post dose groups. * denotes significant difference with controls ($p<0.05$).

References: 1. Le Bihan D. The 'wet mind': water and functional neuroimaging. Phys Med Biol. 2007; 52(7): R57-90. **2.** Saksena S, Rai V, Saraswat VA, et al. Cerebral diffusion tensor imaging and in vivo proton magnetic resonance spectroscopy in patients with fulminant hepatic failure. J Gastroenterol Hepatol. 2008; 23(7 Pt 2):e111-119. **3.** Malik GK, Trivedi R, Gupta A, et al. Quantitative DTI assessment of periventricular white matter changes in neonatal meningitis. Brain Dev. 2008; 30:334-341. **4.** Sotak CH. Nuclear magnetic resonance (NMR) measurement of the apparent diffusion coefficient (ADC) of tissue water and its relationship to cell volume changes in pathological states. Neurochem Int. 2004; 45(4): 569-82.