Tungsten Alloy Based Heavy Metals in Ammunition and Armament May Cause Metabolic Disturbances: A Urinary Metabolomic Approach

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Introduction: Heavy metal–tungsten alloys (HMTAs) are dense heavy metals composed of a mixture of tungsten (91–93%), nickel (3–5%) and either cobalt (2–4%) or iron (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, it is known that at least one alloy, causes rhabdomyosarcomas when fragments are implanted in rat muscle and also nickel and cobalt are known nephrotoxicant and hepatotoxicant. This raises concerns that shrapnel, if not surgically removable, may result in similar tumours in humans. There is therefore a clear need to develop rapid and robust in vitro methods to characterise the toxicity of HMTAs in order to identify those that are most likely to be harmful to human health and to guide development of new materials in the future. In the last two decades, increasing researches have established that metabonomic data are useful for assessment of toxic mechanisms, prediction of toxicity, and identification of biomarkers of disease and toxicity (Nicholson et al., 2002)[1]. It was shown that urine NMR analysis had at least a 4- to 16-fold higher sensitivity than histopathology and clinical chemistry in detecting adaptive and/or toxic changes caused by some compounds (Schoonen et al., 2007)[2].

Aim and Objective: Identification of metabolic biomarker of acute toxicity induced by Tungsten Alloy based heavy metal in rat urine using NMR spectroscopy.

Material & Methods: Male Sprague Dawley rats of 11 weeks of age (n = 6 in each group) and weighing 250-300g were injected with low and high dose of mixtures of tungsten alloy based heavy metal salts ($NiCl_2$, $CoCl_2$ and $Na_2W_2O_4.2H_2O$ prepared in 0.9% saline) intraperitoneally, controls were injected with 0.9% saline only. Urine samples were collected at 8, 24, 72 and 120 h post dose (p.d.) and placed at -80^{0}C till NMR Spectroscopy was carried out. 400µl of centrifuged urine sample was added to 200 µl of deuterated phosphate buffer (pH= 7.4) containing 1mM TSP and transferred to 5mm NMR tube. ^{1}H NMR spectra were acquired at 400.13 MHz, Bruker-AVANCE 400 spectrometer at 298K. 1D NOESYPR pulse sequence was used to achieve satisfactory water suppression on all urine samples. Typically 64 scans were acquired with a relaxation delay of 2 s, flip angle of 90° and spectral width 10 ppm. All data sets were zero-filled to 32K data points and FID was weighted by an exponential function with a 0.3Hz line broadening prior to Fourier transformation. Peak assignment was determined according to previously reported literature[3]. NMR spectra were segmented into region of 0.04 ppm width. The area for each segmented region was calculated and normalized to the total spectral area of each ^{1}H NMR spectrum. In order to discern the presence of inherent similarities of spectral profiles and to order recognize the spectral pattern of endogenous metabolites related to HMTAs based metal salt multivariate analysis using metaboanalyst http://www.metaboanalyst.ca/Metaboanalyst/faces/Home.jsp) was carried out.

Results:- A number of alteration in endogenous metabolites were observed in ^{1}H NMR spectra of urine samples collected at different time point. Figure 1and 2 shows representative NMR urine spectra and PCA plots at 24 h p.d. PCA score plots showed clear separation between control and treated groups. The spectra were dominated by number of metabolites associated with energy metabolism (succinate, citrate, α – Ketoglutarate (α – K.G)), gut flora metabolites (hippurate), osmolytes (taurine), amino acids [Branched chain amino acids (BAA), phenylalanine], N-acetylglutamate (NAG), N-methyl nicotinamide and creatinine. Throughout the study most of the prominent changes in the ^{1}H NMR spectra are related to the endogenous metabolites involved in carbohydrate metabolism such as TCA cycle intermediates, e.g., citrate, succinate and α – K.G. and BAA on low dose group. After 72 h, most of the metabolites returned to normal levels in both the low and high dose group compared to controls, however, changes were still persistent in energy metabolites and BAA.

Disscussion:- Tungsten alloy based heavy metals are known to cause oxidative stress which has been reflected by the partial truncation of TCA cycle in our study. A prominent finding of depletion of the TCA cycle intermediates including citrate, succinate, and α – K.G in p.d. groups at all time points suggests liver damage as these organic acids may be more universal markers for cellular liver pathology (Ishihara et al., 2006)[4]. In addition to this the decrease in the level of citrate might be due to renal tubular acidosis (RTA). The hepatotoxic and nephrotoxic changes induced by tungsten alloy based heavy metals might be due to the presence of nickel and cobalt as seen in our previous study. This has also been supported by pathway analysis showing maximum change in TCA cycle in terms metabolites hits and highest P value in a dose and time dependent manner (Table 1) and VIP score plots showing (VIP > 1.00) for energy metabolites at 72 h (Fig. 3). It has been showed that proximal tubules seem to be the major site of metal-induced nephrotoxicity and the common event in the action of all toxic metals in the proximal tubules cells is attributed to the generation of oxidative stress (Sabolic, 2006)[5]. The increase in the BAA in low dose group might be due to obstacles to the reabsorption of such amino acids and hence aminoaciduria occurred. Aminoaciduria may result from diminished renal reabsorption by the tubules or increased permeability of the glomerular membranes (Macpherson et al., 1991)[6]. It was likely that tungsten alloy based heavy metals may restrain the renal tubule from reabsorbing the BAA, leading to its increased excretion of them in urine. The outcome of the study is that tungsten alloy based heavy metals induce both hepatic as well as renal toxicity and the perturbations can be seen as early as 8 h p.d.

Conclusion:- The major advantage of this study is its simplicity and non-invasive nature because it is based on urine analysis using metabolomics. Overall, these putative metabolic markers could be used to predict the risk of adverse effects by HMTAs. In addition, metabolomics with surrogate metabolic markers can be used as a more rapid and easier preliminary screening tool to determine damage induced by HMTAs.

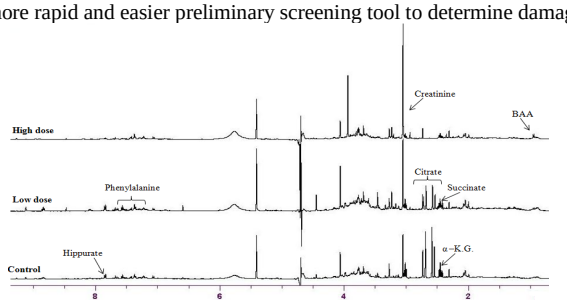

Fig.1 Representative NMR urine spectra from low and high doses of tungsten alloy based heavy metal at 24 h

Time points	Metabolites	Hits	-log(P)	Impact
8h	20	4	14.585	0.174
24h	20	4	9.3	0.174
72h	20	4	12	0.174
120h	20	4	10.267	0.174

Table1. Metabolic pathway mapping of TCA cycle at different time point for low and high dose exposure of tungsten alloy based heavy metals

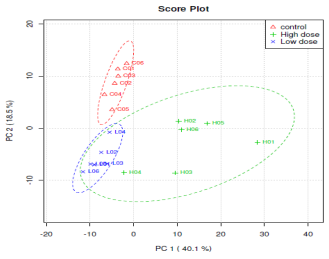

Fig. 2. PCA sore plot based on 1H NMR of urine sample at 24 h from control and low and high dose group of tungsten alloy based heavy metals

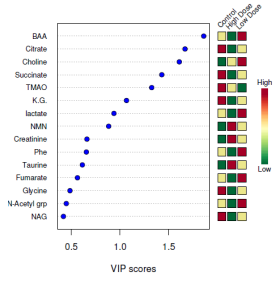

Fig.3. Top 15 significant features of the metabolite markers based the VIP Projection of control and low and high dose group tungsten alloy based heavy metals

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