Toxicological effect of thallium in mice by NMR-based metabolic profiling of urine

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INTRODUCTION: Thallium is a heavy metal that is used in the manufacture of electronic components, optical lenses, semiconductor materials, alloys, gamma radiation detection equipment, imitation jewelry, artist's paints, low temperature thermometers, and green fireworks. Cigarette smoking is also a source of thallium. After absorption, it gets accumulated in liver and kidney and causes renal and hepatotoxicity. Thallium demonstrates at least 5 major toxicologic effects viz: Disruption of potassium-dependent processes, Riboflavin sequestration, Interference with cysteine residues, Ribosomal inhibition and Myelin sheath injury[1]. Biomarkers for effects of thallium intoxication are alopecia, neurological effects, and albuminuria which are indicative of exposure to many other toxicants as well. Therefore, methods are needed for more specific biomarkers for effects of thallium exposure. NMR Spectroscopy is a rapid, non destructive technique that allows the detection of a wide range of structurally diverse metabolites simultaneously, providing a metabolic 'snapshot' at a particular time point. Urine is readily collected and compared with other biofluids, it has relatively low concentrations of proteins and high concentrations of low molecular-weight compounds thus minimizing sample preparation and result in high-quality measurements due to the narrow line widths of the spectral peaks.

Aim and Objective: Identification of metabolic biomarker of acute toxicity induced by thallium sulphate in mice urine using NMR spectroscopy.

Material & Methods: Male A1 strain mice of 10 weeks of age (n = 5 in each group) were injected with thallium sulphate (in 0.9% saline, 5, 10 and 20 mg Tl_2SO_4/kg body weight) intraperitoneally, controls were injected with 0.9% saline only. Urine samples were collected after 3, 24 and 96 hrs post injection and placed at -80^{0}C till NMR Spectroscopy was carried out. 200µl of centrifuged urine sample was added to 400 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 298^{0}K. NMR experiment of single pulse sequence with water prestauration was performed on all urine samples. Typically 64 scans were acquired with a relaxation delay of 2 s, flip angle of 90° and spectral width 15 ppm. All data sets were zero-filled to 32K data points, exponential line broadening of 0.3Hz was applied before Fourier Transform. Peak assignment was determined according to previously reported literature. NMR spectra were segmented into region of 0.04 ppm width. The area for each segmented region was calculated and normalised to the total spectral area of each ^{1}H NMR spectrum. In order to discern the presence of inherent similarities of spectral profiles, an unsupervised pattern recognition method, PCA was conducted on urine samples.

Results: A number of alteration in endogenous metabolites were observed in ^{1}H NMR spectra of urine samples collected after 3 hrs and 24hrs of thallium toxicity but less changes were observed after 96hrs of thallium salt injection (Figure 1). PCA was performed on ^{1}H NMR spectra of urine samples from control and treated animals at all dose levels and the score plot showed a clear separation between control and treated groups along PC2 (Figure 2). There were distinct changes in both the aliphatic and aromatic regions of 3 and 24 hrs spectra compared with control spectra. At 3 hrs, there was increase in the concentration of almost all metabolites observed in ^{1}H NMR spectra viz branched chain amino acids, lactate, ornithine, citrulline, methionine, β-hydroxybutyrate, pyruvate, succinate, citrate, α-ketogluterate, creatinine, Trimethylamine, choline, taurine, ascorbate, allantoin, phenylalanine, N-methylnicotinamide at all doses. However, at 24 hrs, there was a decrease in the concentration of all above noted metabolites in urine samples from mice exposed to higher doses of Tl_2SO_4 (10mg and 20mg /Kg body weight). At 96hrs, all samples tried to reach to control levels except for changes in energy metabolites

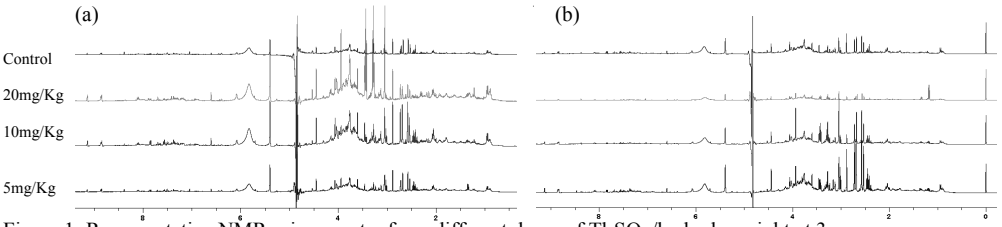

Figure 1: Representative NMR urine spectra from different doses of Tl_2SO_4 /kg body weight at 3 (a) and 24 (b) hrs post injection.

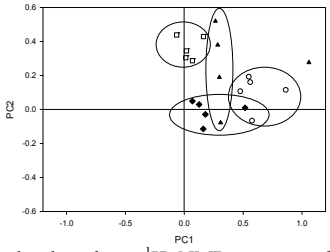

Figure 2: PCA score plot based on ^{1}H NMR spectra of urine sampled on 3 hrs from control mice (○, n = 5) and Tl_2SO_4 group with 5 (□, n = 5) 10 (♦, n = 5) and 20 (▲, n = 5) mg/kg body weight dose

Discussion: Results obtained from the present study clearly points out damage to liver function even at 3 hrs time point in low as well as high doses of Thallium sulfate, however, changes observed were dose dependent. An elevation in the taurine level in urine is the most important NMR biomarker for liver damage and the increased taurine along with increased creatinine have been observed at all three doses at all time points, suggesting liver to be the major target organ of thallium toxicity. Initial damage to liver was also revealed by altered energy and fatty acid metabolism intermediates levels. Whereas damage to kidney function was observed as aminoacidneuria from the beginning of the treatment and decrease in TCA intermediates at 24 hr and 96 hr time point could also be attributed to toxin-induced alteration in tubular acid base status of kidney. Change in N-methylnicotinamide and methionine at all time points suggested glutathione based detoxification reaction occurring in the body for removing thallium from body[2]. Thallium accumulation induced oxidative stress was also noticed in terms of altered aromatic region signals which could be due to disturbances in the gut flora. There are many earlier studies that report of alteration in microflora due to oxidative stress. The outcome of study is consistent with earlier reports on thallium induced hepatic and renal toxicity. However, the most useful part of our study is that metabolic perturbation can also be seen as early as 3 hrs post injection.

Conclusion: NMR-based metabolomics has been proved to be an efficient and valuable technique for the biomarker identification of thallium toxicity, for which only alopecia, neurological effects and albuminuria has been reported till yet. The established 1H NMR technique-based metabolomics approach can provide a systematic and holistic view of the biochemical effect of thallium toxicity on an organism.

References:

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