

NMR spectroscopy based evaluation of urine for identification of changes in functional metabolites on exposure to Thallium-201 in mice

R. Tyagi¹, P. Rana¹, P. Saxsena², M. M. Devi¹, S. Gandhi¹, S. Pal³, and S. Khushu¹

¹NMR Research Centre, INMAS, Delhi, Delhi, India, ²Department of Nuclear Medicine, INMAS, Delhi, India, ³Division and Cyclotron & Radiopharmaceutical Sciences, INMAS, Delhi, Delhi, India

Introduction: Thallium-201 (Tl-201) is routinely administered to people undergoing nuclear medicine scans at activity levels of 3-5 milli curies (mCi)¹. Due to its short half- life, relatively low hazard radiations and rapid biological excretion, thallium-201 has very low radio toxicity when introduced internally. However, the recommended dose of Tl-201 is considered clinical safe and gets excreted from body with in 24-48 hrs of its injection. Physiologically, after injection, it behaves as a potassium analog and gets accumulated rapidly in the cells of many organs, which may lead to some alterations at metabolite levels. NMR Spectroscopy allows the detection of a wide range of structurally diverse metabolites simultaneously, providing a metabolic ‘snapshot’ at a particular time point. Urine in comparison with other biofluids, 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. Therefore, it was proposed to study the metabolic profiles of urine samples obtained from Tl-201 treated mice and look upon the changes at metabolite levels if any using NMR spectroscopy.

Aim and Objective: The present study was performed to assess the alteration, in metabolic profile of mice urine after Tl-201 injection, using NMR spectroscopy.

Material & Methods: Male A1 strain mice of 8 weeks of age (n = 4 in each group) were injected with Tl-201 at dose of 11μCi (equivalent to 3-5 mCi in human) intraperitoneally controls were injected with 0.9% saline. Urine samples were collected after 3 and 24 hrs of Tl-201 injection and stored at -80°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. ¹H NMR spectra were acquired at 400.13 MHz, Bruker-AVANCE 400 spectrometer at 298°K. NMR experiment of single pulse sequence with water pre-stauration 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 ¹H 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 alterations in endogenous metabolites were observed in the urine samples from Tl-201 treated mice compared to controls. PCA was performed on the ¹H NMR spectra of urine samples from control and Tl-201 treated animals and the score plot showed a clear separation between control and treated group at both time points along PC2, with a partial overlap between 3hrs and 24 hrs time point (Figure 1). The prominent changes in endogenous urinary metabolites in Tl-201 treated group, confirmed by inspection of the original NMR spectra and the loading plots comprised an increase in the levels of hippurate, uroconate, phenylalanine, allantoin, citrate, 4-amino hippurate, succinate, β-hydroxy butyrate, branched chain amino acids, ascorbate and citrulline.(Table 1)

	hippurate	uroconate	phenylalanine	allantoin	citrate	4-amino hippurate	α hydroxy isovalerate	Branched chain amino acids	Taurine	succinate	Allantoin	Ascorbate	β- hydroxy butyrate
3 Hrs	↑	↑	↑	↑	↑	↑	↑	↑	↑	↑	↑	↑	↑
24 Hrs	↑	↑	↑	↑	↑	↑	↑	↑	↑	↑	↑	↑	↑

Table 1: Summary of the variations from urine metabolites induced by Tl-201 toxicity

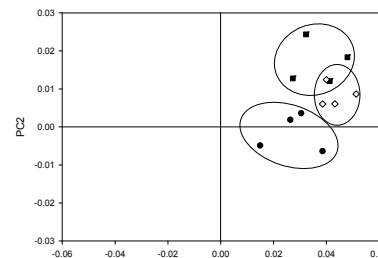


Figure 1: PCA score plot based on ¹H NMR spectra of mouse urine sampled before (●, n = 4) and after 3 (■, n = 4) and 24 (○, n = 4) hrs of Tl-201 injection.

Discussion: Results from the study showed changes in functional metabolites in Tl-201 treated mice after 24 hrs as observed in table -1 and PCA analysis (fig.1). Increased Krebs cycle intermediates (succinate, citrate) and fatty acid metabolism related metabolites (beta hydroxy butyrate, 2 hydroxy isovalerate) represented perturbed energy metabolism and switching over to fatty acid metabolism for energy production. Increased signals from aromatic region of the spectra and hippurate might be due to changes in the gut flora metabolism as radiation released from Tl-201 inside the body could have affected the intestinal gut flora. Minor effect of Tl-201 was also shown on kidney function in terms of increased branched amino acids levels (aminoaciduria) after Tl-201 injection. The γ-radiation and mercury x-rays released by Tl-201 might have produced the free radicals there by causing the oxidative stress. All changes occurring in the Tl-201 treated animals could be the result of oxidative stress induced by free radicals, as free radicals are generated during radiation exposure. Some similar radiation induced changes have also been observed in mice urine samples (submitted as another abstract from our group). Though, the radiation emission is very low in the dose given in the experiment but Tl-201 being a source of internal radiation emission, could have more effect on the physiological functions of the body resulting in some altered functional metabolites. Earlier, some of the changes pertaining to metabolites were also observed in serum of Tl-201 injected mice, but number of metabolites was lesser than that observed in urine². However, changes observed after Tl-201 injection are functional physiological changes which are reversible in both the cases.

Conclusion: NMR-based metabolomics has been proved to be an efficient technique to investigate the perturbation in functional metabolite caused due to Tl-201 and results presented slight liver and kidney dysfunction, which might be associated with an indirect mechanism involving Tl-201 induced oxidative stress. It can be concluded that the dose used for clinical imaging induces only temporary functional metabolic changes due to oxidative stress.

- References:** 1. McKillop JH et al (1980) Thallium 201 scintigraphy (Medical Aledical Progress) West J Med 133:2643.
2. Tyagi et al (2009), Study of thallium 201 induced changes in functional etabolites in mice serum using NMR spectroscopy. Accepted for e-poster presentation at 26th Annual Meeting ESMRMB, 2009