Acute effect of gamma irradiation in mice by NMR based metabolic profiling of urine

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Introduction: Radiation casualty management relies on clinical signs and symptoms, biological assessments, radiation exposure and physical dosimetry. No single assay is sufficient to address all potential radiation scenarios including mass casualties. A quick, non/minimally invasive, multiparameter bioassay strategy based on early response from radiation exposure is needed to adequately assess doses in accidental and occupational overexposure cases. Earlier we have detected NMR spectroscopy based some potential and promising response at metabolite levels in serum samples from mice when exposed to radiation stress[1]. However, for development of non invasive techniques for identification of metabolic markers for radiation related changes in large number of samples, NMR spectroscopy based metabolic profiles of urine is far feasible. Urine spectrum provides a great deal of information by expressing a range of metabolites from energy metabolites to osmolytes and nucleotide metabolites to amino acids. The present study has been proposed to exhibit the time dependent perturbation of metabolism in mice urine due to acute gamma radiation injury.

Aim and Objective: To explore radiation induced alteration in metabolites present in urine in mouse model using high resolution proton NMR spectroscopy for potential application in biological dosimetry.

Material & Methods: Male A1 strain mice of 10-12 weeks of age (n = 5 each) were exposed to radiation dose of 3, 5 and 8 Gy from ^{60}Co source in a GC-220 AECL-CANADA operating at a dose rate of 0.1916 Gy/min and controls (n = 9) were sham irradiated. Urine samples were collected at 6, 24 and 96 hrs post irradiation and stored 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[2]. 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.

Result: 1H NMR Spectra of urine showed significant difference in number of metabolites after radiation exposure. There were distinct changes in both aliphatic and aromatic region of spectra at 6, 24 and 96 hrs sample. However, at 6 h, most of metabolites level were significant different only in mice urine samples irradiated with relatively higher dose (8 Gy) (Figure 1). While few resonances were altered in other two irradiated groups after 6 hrs of irradiation, the spectra were similar to those of control spectra. In animals irradiated with 8 Gy radiation dose, several metabolites were increased after 6 hrs compared to controls including branched amino acids, acetate, citric acid intermediates, tri methyl amine (TMA), taurine, hippurate and aromatic amino acids. After 24 hrs of irradiation, there was substantial increase in taurine, creatinine along with changes in above mentioned metabolites in all three groups. After 96 hrs, NMR spectra showed signs of recovery in animals irradiated with 3 and 5 Gy radiation whereas, levels of creatinine, succinate, 2 oxoglutarate, citrate, hippurate, branched amino acid, aromatic amino acids and acetoacetate were still altered in 8 Gy irradiated animal groups. PCA of the NMR spectra was used to determine whether the high moderate or low dose and control samples could be distinguished from each other in the acute study at different time points (Figure 2). The high dose samples (8Gy) were clustered apart from the controls at all time points. Scores obtained form 3 Gy and 5 Gy group were overlapped at 6 hrs and 96 hrs and could be clustered apart from controls only after 24 hrs of irradiation.

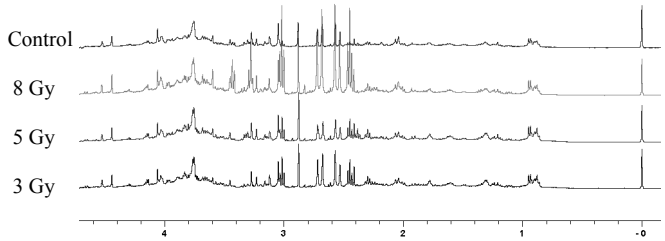

Figure 1: Representative NMR urine spectra from different doses of radiation at 6 hrs post irradiation.

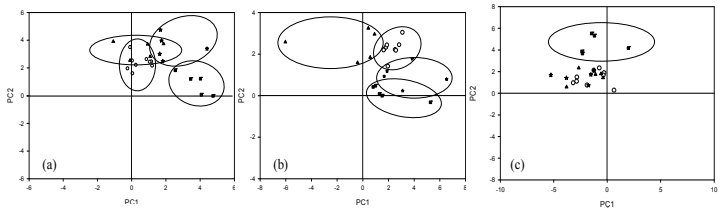

Figure 2: PCA score plot based on ^{1}H NMR spectra of urine sampled on 6 (a), 24 (b) and 96 (c) hrs from control mice (○, n = 9) and irradiated group with 3 (*, n = 5) 5 (▲, n = 5) and 8 (■, n = 5) Gy dose

Discussion: After 6 hrs of irradiation, only mice with 8 Gy radiation exposure exhibited differences in metabolites associated with glucose energy metabolism, protein, fat and gut flora metabolism. Change in key intermediates of krebs cycle, release of amino acid in urine (aminoacidneuria), hippurate, aromatic amino acid, TMA demonstrates high levels of oxidative stress, which markedly enhanced with increasing radiation dose. ROS generated during radiation exposure induce oxidative damage to vital cellular molecules including protein, lipid and membrane and modification of lipids and protein by ROS is implicated in the etiology of radiation induced physiological disorders and diseases. Radiation induced oxidative damage in several organs and mitochondrial membranes have been reported earlier[3]. Radiation induced changes were reflected in urine after 6 hrs but only in higher dose. However, after 24 hrs, these changes appeared in all the three irradiated groups as evident from clear separation of these groups from controls on PCA score plot. PCA plot after 96 hrs of radiation treatment showed returning of 3 and 5 Gy animal groups to the control level. Whereas, animal irradiated with high dose still have physiological perturbations in terms of altered metabolites levels involved with gut flora metabolism, energy and protein metabolism. The observations reported in our study are in accordance with reported literature stating that radiation induced changes tends to recover during latent phase after 3 to 5 days of irradiation depending upon the radiation dose. Our study clearly shows radiation induced damage to physiological systems which increases with increased radiation dose. These physiological disturbances may point out towards multi organ dysfunction. However, correlative studies with clinical biochemical assays will be further helpful in identification of dose related changes.

Conclusion: The present study demonstrates metabolic perturbation of urinary metabolites and gives a non invasive approach to study radiation stress. Such metabolomic techniques help in designing protocols and novel methodologies for mass screening in the event of radiological incident/terrorism.

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3. Kamat J P et al. (2000) J. Ethnopharmacology. 71: 425-435