Exploration of the Effect of Whole Body Ionising Radiation Exposure on the Metabolism of Renal Tissue in Mice Using High Resolution 1H NMR Spectroscopy

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Introduction: Current events throughout the world perceive the growing threat of different forms of harmful exposure of radiological or nuclear attack. In whole body exposure, radiation has an all together effect on complete body at cellular and systemic levels. Acute effects of high-dose radiation appear in the form of haematopoietic cell loss, immune suppression, mucosal damage (gastrointestinal and oral), and potential injury to other sites such as the lung, kidney and central nervous system (CNS). These changes in the metabolic activities of the cells/tissues can directly be reflected in body fluids like blood or urine. There are some published reports, which show altered metabolic profiles in serum and urine using NMR spectroscopy or LC-MS[1, 2]. However, NMR based tissue metabolic studies after whole body exposure to radiation are almost negligible in the literature.

Aim and Objective: The present study was conducted to assess the changes in the metabolite pattern in the renal tissue in mice if any, after a whole body exposure to γ rays.

Material & Methods: Male A1 strain mice of 10-12 weeks of age (n = 6) were exposed to LD_{50} dose of 5 Gy and control (n = 6) were sham irradiated from ^{60}Co source in a GC-220 AECL-CANADA operating at a dose rate of 0.2993 Gy/min. Animals were sacrificed by exsanguinations from cervical dislocation after 5 and 25 days of radiation exposure. Kidneys were excised, washed with phosphate saline buffer and immediately snap frozen in liquid nitrogen. Polar metabolites from kidney tissues were extracted using perchloric acid and the supernatant containing metabolites was lyophilised for 8-10 hours and then dissolved in 550 micro litres of deuterium oxide and transferred to 5mm NMR tube with 1mM TSP (in capillary) as an external reference for spectral acquisition. ^{1}H NMR spectra were acquired on each sample at 400.13MHz on a Bruker Avance 400 spectrometer at 298K. 1D NOESY pulse sequence was used to achieve satisfactory water suppression in the aqueous extracts. For each sample, 64 transients were collected into 32K data points with a relaxation delay of 2 s, flip angle of 90° and a mixing period of 100 ms. A spectral width of 9612 Hz and an acquisition time per scan of 2.56 sec was used. Peaks or multiplets of identified metabolites, including that of internal standard TSP, were integrated to obtain signal intensity. The metabolite concentrations were calculated as described by Sharma et al[3]. Metabolites concentration was compared in control and experimental animals using one way ANOVA.

Results: ^{1}H NMR spectra of control aqueous extracts showed signals from a range of organic acids, amino acids, sugars, and glycogen. In the ^{1}H NMR spectra of tissue samples, the irradiated group did not exhibit significant alteration in the concentration of most of the metabolites except succinate identified in the spectra after 5 days of radiation exposure when compared with controls (Table 1). After 25 days of radiation treatment, ^{1}H NMR spectra of tissue extracts samples exhibited statistically significant increase in most of the metabolites, viz, branched amino acids, β hydroxybutyrate, lactate, alanine, lysine, choline and myo-inositol. However, succinate, TMAO, betaine and taurine did not show any significant difference between controls and irradiated mice (Table 1).

Discussion: After five days of radiation, no significant changes in metabolite concentrations were observed in irradiated mice group except for succinate. Decreased concentration of succinate reflects a decrease in the oxidative catabolism thereby leading to some kind of energy deprivation. However, marked effect on the metabolism of kidney tissues by radiation exposure was seen only after 25 days of the radiation. It supports the statement that kidney is among the most radiosensitive late responding critical organs and radiation damage develops slowly[4]. Increased lactate content in irradiated mice kidney tissue suggested an increase in the rate of anaerobic glycolysis. It has been stated in earlier enzyme based studies that anaerobic glycolysis is not only unaffected by the radiation but may be capable of an increased activity after exposure to radiation[5]. This increase in anaerobic pathway could be due to decreased oxidative metabolism as shown by decreased concentration of succinate during early effect (after 5 days) studies. The alterations in the amino acid concentrations indicate decreased protein synthesis or increased protein degradation or both. Kidney cells are the site of degradation of branched amino acid and play an important role in amino acid homeostasis. Increased amino acid content in the extract suggests changes in ion transport and points to altered amino acid metabolism[5]. Beta hydroxy butyrate was found in higher concentration in the kidney tissue since it's an end product of branched amino acid (Ile, leu) degradation. Increased concentration of glutamate could be due to stress induced renal acidosis or disruption of tricarboxylic acid cycle.

Metabolites	Control	Irradiated group (after 5 days)	Irradiated group (after 25 days)
Ile+Leu+Val	2.62±0.61	2.97±1.94	6.12±0.15*
β hydroxy butyrate	4.99±1.29	4.35±4.22	9.56±1.73*
Lactate	25.89±4.07	18.73±10.98	42.06±5.35*
Ala	11.71±2.33	13.35±7.31	19.70±2.50*
Lys	25.60±5.70	21.37±10.85	45.67±7.74*
Gln/Glu	18.85±4.17	15.63±8.25	31.78±4.57*
Glu	8.61±2.16	7.13±3.93	13.99±1.65*
Succinate	6.12±1.45	4.30±1.80*	7.02±0.83
Choline	6.61±1.39	6.41±4.02	13.87±3.77*
TMAO+Betaine	1.44±0.32	1.29±1.40	1.94±0.39
Taurine	21.54±3.97	15.65±9.57	30.65±5.36
Inositol	27.73±4.46	22.42±14.63	49.77±10.18*

***Significant at 0.05 level**

Table 1: The concentration of various metabolites (μmol/g wet weight) from PCA extracts of kidney tissue obtained from controls and irradiated mice after 5 and 25 Days along with the p-value

Conclusion: Overall, NMR spectroscopy shows radiation induced altered metabolic status of kidney tissue through changes in low molecular weight important metabolites. This approach can further be helpful in correlation with other bio analytical techniques identification of potential biomarkers for organ specific dosimetry. Moreover, this work along with NMR study of urine metabolites could give a comprehensive profile of metabolites getting altered during radiation exposure.

References:

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