NMR based metabonomic investigation on the biochemical effects induced by radiation exposure in mouse serum

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Introduction: With the growing need for nuclear waste disposal into the environment and the ever-increasing threat of a terrorist nuclear event, it is now necessary to explore biomarkers of ionizing radiation exposure that can be used for mass screening in the event of a radiological incident. Analysis of biofluids such as serum, urine is the simplest and minimal invasive approach to work at the mass screening level. Radiation stress causes metabolic, physiological alterations as well as neuropsychological disorders in normal humans and animals, which may directly be reflected in blood or serum, since it provides a unique window to look into the biochemical status of living organism. NMR Spectroscopy allows the detection of a wide range of structurally diverse metabolites simultaneously, providing a metabolic 'snapshot' at a particular time point[1]. Serum contains nearly all of the low-molecular weight metabolites, and the ^{1}H NMR spectra of serum from animals under similar physiological conditions are highly reproducible.

Aim and Objective: The study has been planned to find radiation induced changes in metabolites present in serum sample in mouse model using high resolution proton NMR spectroscopy.

Material & Methods: Male A1 strain mice of 10-12 weeks of age were exposed to LD_{50} dose of 3 and 5 Gy and control (n = 6 in each group) were sham irradiated from ^{60}Co source in a GC-220 AECL-CANADA operating at a dose rate of 0.2993 Gy/min. Blood samples (~ 500µl) were collected by puncturing the retro-orbital vein after 5 days of radiation exposure. Serum samples of 200µl obtained from blood were mixed with 400 µl of D_2O and transferred to 5mm NMR tube with 1mM TSP (in capillary) as an external reference compound for spectral acquisition. ^{1}H NMR spectra were acquired on each sample at 400.13MHz on a Bruker Avance 400 spectrometer at 298K. Water signals and broad protein resonances were suppressed by a combination of presaturation and the CPMG pulse sequence (τ = 200µs, n = 500). For each sample, 64 transients were collected into 32K data points with a relaxation delay of 2s, spectral width of 9612 Hz and an acquisition time per scan of 3.98 sec. 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[2]. Metabolites concentration was compared in control and experimental animals using one way ANOVA.

Result and Discussion: The ^{1}H NMR spectra of serum of irradiated groups exhibited an altered biochemical composition, after 5 days of exposure to gamma radiation with respect control (Table 1). Mice group irradiated with 5Gy dose (Group III) showed more significant changes in the metabolites as compared to Group II (3 Gy). Decrease in serum lactate and increase in creatine levels in both the groups suggested an increased energy demand, increased gluconeogenesis and a metabolic switch towards energy conservation. There was radiation induced enhanced protein breakdown and amino acid metabolism in irradiated animals as evident from increased free branched amino acids levels (BAA) found in the serum samples. However, significant difference was observed in animals exposed with slightly higher dose (5Gy). Interestingly, a very prominent signal of propionate was observed in irradiated mice samples. Since, propionate is an intermediary metabolite of amino acid catabolism and enhanced breakdown of protein might have resulted in increased concentration of propionate and other metabolites. Few earlier studies have shown an increase in propionate level in blood plasma after exposure to ionising radiation[3,4]. No difference could be obtained in glutamine/ glutamate (Glu/Gln) levels. Significant decrease in choline levels were observed in both irradiated groups. Direct attack of free radicals generated during radiation causes membranal damage resulting in changes in choline and lipid levels. Choline is a common polar head group of membrane phospholipids. Decrease in choline levels could be due to up regulation of repairing process of cell membrane, which utilise choline for synthesis of phospholipids[5]. Prominent increase in the levels of LDLs or VLDLs (not shown in table) was observed in the spectra, which could be because of altered cholesterol metabolism resulting in increased concentration of LDLs in plasma[6].

S. No	Metabolites	Control	Group II (3Gy)	Group III (5Gy)
1.	Branched Amino Acid	0.216±.096	0.271±0.051	0.329±0.035*
2	Alanine	0.409±0.174	0.518±0.098	0.638±0.163*
3.	Propionate	1.566±0.290	7.153±1.484*	7.206±1.780*
4.	Choline	0.701±0.147	0.552±0.171*	0.558±0.080*
6.	Creatine	0.146±0.066	0.230±0.091*	0.219±0.058*
7.	Glu/Gln	0.263±.181	0.381±0.174	0.279±0.032
8.	Lactate	11.726±2.043	7.988±1.75*	8.507±1.152*
9.	Pyruvate	0.180±0.085	0.203±0.058	0.151±0.054

***Significant at 0.05 level**

Table 1: The concentration of various metabolites (µmol/g wet weight) from serum samples obtained from controls and irradiated mice after 5 days

Conclusion: The present study helps to explore the metabolic alteration in serum and reflects the radiation induced changes in the metabolism of different tissues. However, with the use of other biochemical, analytical and molecular approach along with multivariate analysis, this NMR based metabolomic study will work as a platform for developing strategies for minimal invasive radiation biodosimetry

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