High Resolution MR Spectroscopy Reveals Radiation Induced Metabolic Changes in Mouse Liver Tissues

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Introduction: Liver is considered to be a highly radio-resistant organ on the criterion of morphological changes, radiation hepatitis and radiation necrosis of the liver. However, whole body radiation exposure may induce a variety of additional effects that can be expressed at cellular and tissue levels like generation of oxidative stress, alteration in gene transcription or changes in signal transduction, which ultimately has cumulative effect on the metabolic status of cell or tissue. High resolution ^{1}H NMR spectroscopy is established as a tool for investigating the metabolic consequences of drug as well as environmental toxicity. Therefore, present study has been planned to use NMR spectroscopy technique to uncover metabolic response of liver in mice when exposed to a moderate dose of ionising radiation.

Aim and Objective: To study radiation induced changes in the metabolic profiles of hepatic tissue in mice using NMR Spectroscopy.

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. Liver was excised, washed with phosphate saline buffer and immediately snap frozen in liquid nitrogen. Polar metabolites from liver tissues were extracted using perchloric acid and the supernatant containing metabolites was lyophilised for 8-10 hours and then dissolved in 550 µl of deuterium oxide 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. 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 2s, a 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[1]. 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 and bases, amino acids, sugars, and glycogen. The concentration of metabolites determined in the tissue extract of control and irradiated group after 5 and 25 days of radiation are shown in Table 1. The irradiated group did not exhibit any significant alteration in any of the metabolites identified in the spectra after 5 days of exposure to radiation when compared with controls except for choline (Table 1). However, there was remarkable increase in the concentration of all metabolites observed in the ^{1}H NMR spectra after 25 days of radiation. Concentration of metabolites increased almost three to four times when compared with controls or with irradiated mice after five days.

Discussion: After five days of radiation, no significant changes except for choline were observed in irradiated mice group supporting the statement that liver is a moderately sensitive and late responding critical organ and radiation damage develops slowly[2]. However, marked effect on the metabolism of liver by radiation exposure was seen only after 25 days of the radiation. Liver is the site of all non toxic disposal of amino group through aminotransferase activity (GPT and GOT). Increased alanine and glutamate levels in irradiated group could be due to inhibition of transamination by radiation. Increased levels of branched amino acid (Ile, Leu, Val) could be result of enhanced protein degradation. Radiation exposure induces free radical formation which directly attacks the phospholipid present in the membrane resulting in damage of membrane structure. Since most of the branched amino acids are non polar and are important constituents of hydrophobic region of membranal protein, which may get released during damage of cellular membrane. Increased choline and glycerophosphoryl choline (GPC) levels in the liver extract could also be the result of membranal damage. Increased lactate content in irradiated mice liver tissue suggested decrease in gluconeogenesis as lactate and alanine are common precursors of gluconeogenesis. However, increased energy demand in the liver cells is usually met with the acetylCoA produced from TGLs of fat. Since fat/lipid metabolism is influenced during radiation exposure and some of the earlier studies have suggested altered cholesterol metabolism resulting in increased concentration of LDLs in plasma[3]. We have also found raised LDLs and VLDLs in serum of irradiated mice (data presented in other poster). Increased β hydroxybutyrate could also be result of increased fatty oxidation, since acetylCoA obtained from fatty acid catabolism could undergo conversion of ketone bodies.

Metabolites	Control	Irradiated Group (5 Days)	Irradiated Group (25 Days)
Ala	10.737±2.250	14.749±0.787	40.577±2.787*
Asp/lys	14.470±1.112	23.593±12.885	53.996±5.272*
Ile+Leu+Lys	2.269±0.548	3.755±0.123	7.238±1.248*
β hydroxybutyrate	3.422±0.815	2.461±4.263	11.244±2.419*
Choline	1.548±0.335	3.312±0.620*	7.308±1.971*
Gln/Glu	11.004±1.158	15.154±5.878	37.126±0.522*
Glu+GSH	8.539±0.858	9.617±1.114	29.633±2.556*
GPC	4.231±0.211	4.769±1.435	12.618±2.395*
Lactate	44.517±6.815	44.593±9.145	78.3057±5.709*
Lys	16.686±2.228	25.557±2.582	45.5143±9.180*
Succinate	7.812±0.357	8.532±0.936	28.029±1.731*

Conclusion: This is a preliminary work in the area of radiation induced biochemical changes using high resolution NMR spectroscopy with small sample size and moderate dose of radiation. Our results have shown that radiation certainly has some impact on liver metabolism and NMR spectroscopy has remarkable potential in revealing the changes at metabolite level. However, use of other biochemical and molecular approaches will further be helpful in substantiating our results and for development of potential biomarkers.

References:

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3. Feurgard et al (1999) Intl. J Radiat. Biol., 75: 757-766.

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 ***Significant at 0.05 level**