High resolution 1H NMR spectroscopy reveals acute changes in hippocampal metabolic physiology in a mouse model for cranial irradiation

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Target Audience: Researchers, Clinicians and Students

Introduction: Of all the brain regions, hippocampus is known to be very sensitive to radiation exposure, in particular CA1 and subgranular zone regions (SGZ). Hippocampus associated functions learning and memory are significantly affected due to suppression of hippocampal neurogensis by radiation induced oxidative stress. Recently few reports have shown impaired neurogenesis, increased hippocampal neuronal apoptosis and reduced growth hormone secretion within 24-48 hrs of radiation exposure. There is a need for holistic investigation of radiation induced change in hippocampus during early acute phase. Since, these acute changes could be considered important and might play a significant role in early delayed and late delayed effects. Current knowledge on the early metabolic response of hippocampus for radiation exposure is still fragmented. To attain comprehensive metabolic information of hippocampus post irradiation, high-throughput techniques is prerequisite. High resolution Nuclear Magnetic Resonance (NMR) spectroscopy is one such technique that is often employed to provide a complementary metabolic fingerprint of biological system.

Aim of the study: The study was designed to look for comprehensive metabolic changes using NMR based metabolomic approach. The study highlights the significance of high-throughput analysis of acute radiation exposure effects on brain metabolic physiology.

Materials and Methods: A total of 18 strain 'A' male mice (8 to 10 weeks old) were taken and randomly divided into two groups of which twelve animals in the first group were exposed to single dose of 8Gy cranial radiation through Tele ^{60}Co irradiation facility unit, rest of the animals (n = 6) served as sham irradiated controls. Animals were sacrificed and hippocampus was dissected out carefully at day 5 and 10 post irradiation in radiation group. Control animals (n = 6) were also sacrificed and hippocampus was dissected. The polar metabolite extracts of dissected hippocampus (50-70mg) was obtained based on acetonitrile extraction method. The tissue extracts were reconstituted in 480 µl of D2O containing 1mM TSP as an internal NMR chemical shift standard and 1H NMR spectra were acquired at 400.13 MHz on a Bruker 400 MHz spectrometer at 300 K using 1D ZGPR pulse sequence. For each sample, 128 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 6410.256 Hz and an acquisition time per scan of 5.11s were used. Each ^{1}H NMR spectrum from tissue was integrated into regions with equal width (0.04 ppm) using AMIX software package (Bruker Germany). The data set was normalized, mean centered and pareto scaled before performing the Principal component analysis (PCA) and Partial least square-discriminant analysis (PLS-DA) processing using Metaboanalyst 2.0 (http://www.metaboanalyst.ca/Metaboanalyst/faces/Home.jsp)).

Results: The ^{1}H NMR spectra displayed several metabolites that were altered in the brain after 8Gy cranial irradiation compared to controls (Figure1). The metabolites observed in 1H NMR spectra of hippocampus were mainly associated with energy metabolism, osmolytes, excitatory neurometabolites, N Acetyl Aspartate (NAA), ketone metabolism and membrane metabolites. The score plot based on PCA analysis of full binned region showed irradiated and controls as distinct well separated clusters at both the time points. 2D score plot of principal component1 (PC1) and principal component 2 (PC2) showed a total variance of 72%. According to the loading plot of PCA, there were 18 metabolites identified as associated with metabolic response post radiation exposure. Significant decrease in GABA, glutamine, glutamate, succinate, citrate, choline and GPE was observed in irradiated animals compared to controls at both the time points. On the other hand, aspartate, acetate, ketone and scyllo-inositol were strikingly elevated compared to controls both at day 5 and 10 compared to controls (Figure 2). Most of the identified metabolites based on PCA analysis were mainly associated with energy or related metabolism. PLS-DA analysis also showed good classification of irradiated groups at both the time points with accuracy of 1.0 (R2=0.99, Q2=0.92)(Figure 3). Similar to PCA analysis observations, PLS-DA and VIP score also showed energy metabolites and ketone bodies as the main metabolites that influenced the separation of irradiated group from controls.

Discussion: The results indicate an overall decrease in hippocampal metabolic activity due to irradiation and provide strong evidence that hippocampus is metabolically responsive to irradiation. Reduced TCA cycle intermediates in the present study indicated oxidative stress induced impairment of TCA cycle that may eventually lead to disturbed energy metabolism. Reduced glutamate level and elevated aspartate level in hippocampus post irradiation in our study further substantiate activation of partial TCA cycle by utilising glutamate as an energy source and feeds the cycle via a-ketoglutarate during oxidative stress. As Glu/Asp carrier irreversibly transports a proton and glutamate in exchange for Asp across the inner mitochondrial membrane through malate aspartate shuttle. Increased aspartate is further complemented with decreased levels of citrate observed in our study. Since more of OAA would be diverted towards aspartate synthesis resulting in lesser availability of it for citrate synthesis in TCA cycle. Increased acetate and acetone levels in irradiated groups compared to controls in our study indicate elevated ketogenic metabolism that further complements the change in the availability of energy substrate. The significant decrease in the replenishment of some of TCA cycle internediates and significant increase in ketone bodies post irradiation might be related to radiation induced poor glucose metabolism. Our present study supports that ketone bodies metabolism as an alternative energy source might have been permitted to meet the metabolic demand of brain post irradiation. Altered energy metabolism in hippocampus also showed its effect on GABA levels. GABA, which is an inhibitory neurotransmitter, is synthesized from glutamate. Though changes were observed in many metabolites, sustainable changes were associated with oxidative stress induced altered energy and related metabolism.

Conclusion: 1H NMR spectroscopy along with multivariate analysis revealed a general decline in the metabolic activity of irradiated hippocampus compared to controls. This decline is characteristically manifested in the energy metabolism and glutamate/glutamine/GABA metabolism. The changes observed in hippocampus metabolism during acute phase of radiation injury might have long lasting effects on cognitive development and function. However, further studies are required to correlate these changes with neurobehavioral and structural altered to understand the effect of radiation on cognitive dysfunction.

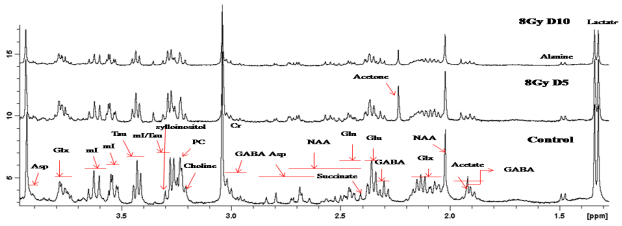

Figure 1: comparative spectra of irradiated groups at day 5 and 10 and controls

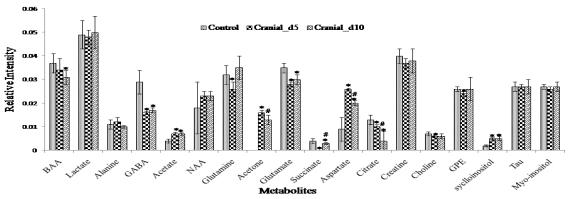

Figure 2: comparative graphs of metabolites at both time points

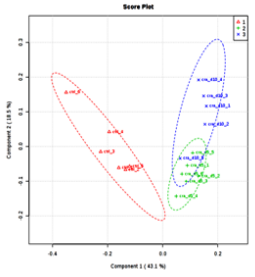

Figure 3: PLS score plot of irradiated group and controls