

Quantitative MR Spectroscopic Evaluation of Radiation-Induced Brain Injury in a Rat Model

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INTRODUCTION

Brain irradiation is known to cause long term functional deficits in patients with radiotherapy. Recent data suggested that progressive dementia occurs in approximately 20-50% of brain tumor patients who are long-term survivors after treatment with whole brain or large field irradiation [1]. While it is well known that ionizing radiation exposure to the brain can lead to immediate oxidative damage at cellular levels [2, 3], little has been understood about long term effects and its mechanism to normal tissue damage. *In vivo* localized MRS offers a tool to non-invasively study the metabolic and neuro-chemical changes throughout radiation treatment, which could help determine bio-markers that are relevant to normal tissue injury. This could also lead to the development of an accurate noninvasive imaging procedure to detect the neuro-functional changes at early stages. The specific aim of the current study was to detect and determine, using MRS, the long term neuro-chemical changes in rat brain associated with exposure to a clinically relevant dose of irradiation.

MATERIALS AND METHODS

MR experiments were conducted on 30 adult male (66 weeks old) Fisher 344 rats. Twenty rats underwent whole brain irradiation, with a total dose of 45 Gray (Gy) from a Cesium irradiator, distributed over 9 separate 5 Gy fractions. The irradiations were started when the rats were 19 weeks of age, and were delivered over a 29 day period. The other 10 rats served as controls. MR experiments were performed on a 7T small animal MRI system (Bruker Biospin, Ettlingen, Germany), equipped with an actively-shielded gradient set, with a maximum gradient strength of 400 mT/m. Signal excitation and reception were accomplished with a linear birdcage coil and a surface coil, respectively. Conventional T₂-weighted RARE images (TE = 41.40 ms, TR = 3000 ms, slice thickness = 1 mm, matrix = 256 x 256) were acquired in coronal (FOV = 3.2 x 3.2 cm²) and axial (FOV = 3.5 x 3.5 cm²) orientations to assess differences in anatomy and to assist in positioning of the MRS voxel. Spectra were obtained with a double spin-echo (PRESS) sequence (TE = 16.2 ms, TR = 1718.5 ms) with and without VAPOR water suppressions. Data were acquired at a spectral width of 4 KHz, and 256 repetitions were averaged to create each individual water-suppressed spectrum. All 1st and 2nd order shim coil currents were adjusted using the FASTMAP technique [4]. Identical voxels (5 x 5 x 5 mm³) were prescribed in a central location of the forebrain, below the cortex, to include tissue from both the striatum and hippocampus. The individual spectra were processed and quantified using LC Model [5].

RESULTS

Satisfactory spectra were obtained from rat brains for both the control and irradiated groups. Typically, before each unsuppressed water reference scan, each localized voxel was shimmed until the water peak had a linewidth (full-width at half-maximum) of < 10 Hz. Table 1 lists the results of quantified metabolite peak ratios (proportional to the metabolite concentrations) for Choline-containing compounds (Cho), N-Acetylaspartate + N-Acetylaspartylglutamate (NAA+NAAG), Glutamate + Glutamine (Glu+Gln), *myo*-Inositol (mI), Taurine (Tau) and γ -Aminobutyric acid (GABA) to Creatine + Phosphocreatine (Cr+PCr). The ratios of Cho/Cr+PCr, NAA+NAAG/Cr+PCr and Glu+Gln/Cr+PCr all increased in the irradiated rats. However, the ratio of mI/Cr+PCr decreased in the irradiated rats, and the ratios of Tau/Cr+PCr and GABA/Cr+PCr showed no significant changes. The changing metabolites, along with statistical analysis, are all illustrated in figure 1.

Metabolite Ratios	Control (m ± sd)	Irradiated (m ± sd)
Cho / Cr+PCr	0.24 ± 0.01	0.28 ± 0.03
NAA+NAAG / Cr+PCr	0.96 ± 0.16	1.21 ± 0.15
Glu+Gln / Cr+PCr	1.59 ± 0.14	2.05 ± 0.29
mI / Cr+PCr	1.02 ± 0.09	0.86 ± 0.12
Tau / Cr+PCr	0.48 ± 0.09	0.39 ± 0.19
GABA / Cr+PCr	0.43 ± 0.12	0.43 ± 0.19

Table 1: Summary of metabolite concentration ratio measurements

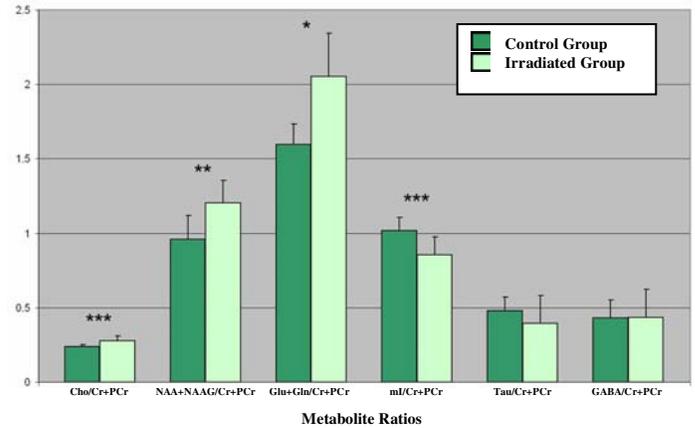


Figure 1: Comparison of metabolite concentration ratios for control and irradiated groups (* P < 0.001, ** P < 0.005 and *** P < 0.01)

DISCUSSION

The statistical correlation between the metabolite concentration ratios shown above indicate that Cho, NAA+NAAG, Glu+Gln and mI signals all may serve as sensitive metabolic markers related to the late effects of brain irradiation, while the changes in Tau and GABA signals are much less indicative. The Glu+Gln signals offer the most significant changes when comparing the two groups of rats; however, the standard deviations of the Glu+Gln measurements are higher due to spectral overlapping. The NAA+NAAG signals may offer the most sensitive marker with respect to alterations in neuronal function due to the long term effects of irradiation damage. As the dominant peak in the spectra, the NAA+NAAG signals are both accurate in measurement and significant in the amount of change between the two groups. However, the underlying mechanism related to the significant composite NAA+NAAG signal increase in 12 months post-irradiated rats has yet to be determined. Among several possibilities, mitochondrial dysfunction related to brain irradiation could be the major contributor to the elevated NAA+NAAG signals in the study group. As for the other metabolites, the small increase in the Cho signal in the irradiated rats could be indicative of a small increase in cell proliferation that often follows cell loss due to ionizing radiation damage. Also, the decrease in the mI signal in the irradiated rats is most likely due to a radiation-induced breakdown of intracellular signaling pathways. The exact mechanism of all these changes is still unclear and will require further studies to correlate *in vivo* and *in vitro* results.

REFERENCES

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