Abstract

Purpose To investigate the neuronal response to ischemic injury following exposure to whole brain proton irradiation.

Methods Brain only proton irradiation (8 Gy, 250 MeV) was performed ten days prior to middle cerebral artery occlusion (MCAO) in 1 year old male Sprague Dawley rats. MCAO was induced in two animal groups: proton irradiated (MCAO + Rad) and MCAO only. Magnetic resonance imaging (MRI) and quantitative analysis were performed prior to and 2 days after irradiation, and then 2, 14 and 28 days after MCAO. After the last imaging time point animals were sacrificed and TUNEL staining was performed on 4% paraformaldehyde – fixed brain sections.

Results Neuroimaging demonstrated a reduction in ischemic lesion volume in the MCAO + Rad group compared with MCAO alone. Neurological deficits did not differ between ischemia groups. Interestingly, there was a 34% decrease in the number of TUNEL-positive cells in MCAO + Rad brains compared to MCAO alone.

Conclusion Our results suggest that radiation treatment reduces brain edema, ischemic lesion volume and peri-ischemic apoptosis. The underlying mechanisms are currently unknown and additional studies will elucidate the significance of these results.

Keywords Cerebral ischemia • MRI • MCAO • proton • radiation • stroke

Introduction

Radiation has been shown to alter normal brain physiology, including brain microcirculation (8). Radiation-induced damage results in pathological modification of blood vessels, cortical atrophy, cerebral white matter necrosis and fibrosis, and delayed (months to years) neurological deficits (19). However, radiation is used clinically for treatment of brain cancers and arteriovenous malformations (AVM) (6, 18). Modern radiotherapy techniques allow minimal damage to surrounding tissues thus improving patient survival and outcomes (12). Yet, more attention is needed to evaluate long-term treatment-related morbidity. Radiotherapy accelerates cerebral vascular atherosclerosis (5), seriously impairs cognitive functions (3, 10, 13), and leads to increased risk of stroke even many years after the initial therapy (12). Child cancer survivors who had undergone radiotherapy are at significantly increased risk for adverse cardio- and cerebrovascular effects (11). The risk of developing stroke, along with increased risk of vasculopathy, ‘blood clots’ and ‘angina-like symptoms’, is more than 40 times greater among childhood brain-tumor survivors than sibling controls (1).

While high doses of radiation are known to exert pro-inflammatory effects, low doses have been shown to have an anti-inflammatory effects (15). Inflammation significantly contributes to the acute brain damage and subsequent tissue loss caused by ischemia/reperfusion (17). We hypothesized that a moderate dose of proton radiation may have beneficial effects on post-ischemic brain injury. The mechanisms underlying the neurorepair process after combined radiation and ischemic injury are unknown and knowledge of these
mechanisms may improve therapeutic approaches in patients exposed to radiation and at risk of stroke.

**Methods**

A total of 10 male, 1 year old Sprague Dawley rats (530–550 g; Harlan, Indianapolis, IN, USA) were divided into MCAO and MCAO + Rad groups (n = 5 in each group). Animals were housed under 12:12 h light/dark cycle with access to water and food ad libitum. All procedures were approved by the Animal Care and Use Committee at Loma Linda University (LLU).

In the MCAO + Rad group, brain only irradiation was performed at 10 days (Day -10) before middle cerebral artery occlusion (MCAO) (Day -0). Irradiation was performed at the LLU Proton Treatment Facility with accelerated protons (8 Gy; 250 MeV; 5.8 Gy/min; collimated, brain only). The focal ischemic insult was induced by 50 min MCAO using an intraluminal thread technique (7). Neurological tests were performed at 1, 2, 14, and 28 days after ischemia induction by a blinded investigator using an 18-point neurological scoring system (4) (minimum neurological score was 3 and the maximum in healthy animals was 18).

MRI data were collected prior to irradiation and 2 days after irradiation (Day -8), and after MCAO induction (2, 14, 28 days). MR data was obtained using a Bruker 4.7T and analyzed as previously described (2). 3D volumes and ROI analysis for T2WI were obtained and summarized.

After the final imaging time point, animals were intracardially perfused with ice-cold 0.12 M Milloning’s phosphate buffer, pH 7.3 (1 mL/1 g body weight) and fixed in 4% paraformaldehyde (Electron Microscopy Science, Hatfield, PA). Brains were cut coronally (30 μm) through the ischemic core (based on MRI) on a cryostat.

Terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) was performed on coronal sections from each animal at the level corresponding to the peri-ischemic region (approximately 1 mm from ischemic core) by using in situ Cell Death Detection Kit, Fluorescein, according to the manufacturer’s instructions (Roche Indianapolis, IN) (9).

Data are expressed as the mean ± SEM. Statistical significance (p < 0.05) was tested by a Student’s t-test or ANOVA followed by Bonferroni post hoc comparisons.

**Results**

**Neurological Function**

MCAO resulted in significant neurological deficits in both animal groups compared to pre-ischemia. However, there were no significant differences in neurological deficits detected between MCAO and MCAO + Rad groups at all time points after ischemia induction.

**MRI**

Neuroimaging (T2WI) of the ischemic tissue demonstrated that whole brain radiation exposure of MCAO + Rad animals resulted in a significant reduction of the ischemic lesion volume (*p < 0.05 vs MCAO, ANOVA) (Fig. 1a, b). T2 values (indicative of brain water content) in MCAO and MCAO + Rad groups were significantly increased within the lesion volume in both groups after MCAO induction compared to pre-stroke values.

**TUNEL Staining**

Quantification of the number of TUNEL positive cells detected a 33.7% decrease in the peri-ischemic area (1 mm
Radiation Exposure Prior to Ischemia Decreases Lesion Volume, Brain Edema and Cell Death

animals (*p < 0.001 vs MCAO, t-test) was significantly reduced in MCAO + Rad compared with MCAO only percentage of TUNEL positive cells detected in the peri-ischemic area from core of ischemia) in MCAO + Rad animals compared with MCAO (p < 0.05 vs MCAO, t-test) (Fig. 2).

Discussion

Radiation treatment has a long clinical history but the biological effects of this treatment are still debated. Radiation is an effective treatment modality for tumors, angiomias, arteriovenous malformations or therapy of resistant pain syndromes such as trigeminal neuralgia (14, 16). Radiation is considered the first line of defence against some tumor types but there is no unanimous opinion about potential adverse effects of treatment including consideration of long-term outcomes.

The model presented in our study was designed to investigate the effects of combined radiation and ischemic injury, similar to that which might be encountered in the clinical population. We observed reduced lesion volume as well as decreased TUNEL positive cells in radiation treated animals, indicating that apoptotic cell death was significantly reduced in ischemic animals exposed to radiation.

Our results suggest that radiation exposure modified outcomes of experimental ischemic stroke similar to that seen in the human patient population. We present data from a relatively short time period after radiation (38 days) and ischemia (28 days). Long-term studies are needed to understand outcomes and underlying mechanisms of presumed neuroprotection.

Conflicts of interest statement We declare that we have no conflict of interest.

References

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