Radiation pretreatment result in a dramatic increase in ADC after ischemic brain injury in rats

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Introduction: It has been shown that radiation affects normal brain physiology, including brain microcirculation. Different radiation doses are widely used for brain tumor and angiovascular therapy and there is anecdotal evidence of a serious risk of stroke among patients even many years after the initial treatment. Recently in both human and animal studies inflammation appears to significantly contribute to the acute ischemia-reperfusion brain injury and a subsequent tissue loss. It is a well established that radiation has a dose dependent immunosuppressive effect. However, it is still unknown how after radiation therapy the brain responds to a focal insult such as stroke. The goal of our study is to investigate how a single relatively small dose of brain irradiation can alter the brain's repair mechanisms after ischemia/reperfusion injury.

Methods: Animals. Middle cerebral artery occlusion (MCAO; 50 min) was induced in 10 male, 1 year old Sprague Dawley rats (540 – 580g), among them 5 proton irradiated (MCAO+Rad) and 5 control (MCAO only) animals. Neurological tests were performed at 1, 2, 14 and 28 days after ischemia induction. At 28 days after MCAO animals were sacrificed and TUNEL staining was performed. Local brain irradiation: Ten days prior to MCAO rats were exposed to 8 Gy brain only 250 MeV/amu protons. Neuroimaging and Analysis: All animals were imaged using diffusion weighted imaging (DWI) and T2WI to monitor ischemic injury progression in an 11.7T scanner (Bruker, Bruker Biospin) and analyzed using Cheshire v4.3, Hayden Image (Parexel International Corp.) software. Analysis consisted for 3D volumetric reconstructions of whole brain, and ischemic tissue volume using Amira (Mercury Computer Systems) software.

Results: Neuroimaging (DWI and T2WI) (Fig 1) of the ischemic tissue demonstrated that brain radiation pretreatment caused in a significant reduction in ischemic lesion volume (Fig. 1A-C). 3D volumetric assessment clearly shows a smaller ischemic lesion at all time points compared to MCAO only animals. Increased water mobility (ADC values) (Fig.2) was observed at all time points in radiation exposed animals when compared to ischemia only. T2 values had a time dependent reduction in MCAO+Rad animals compared with MCAO only (16.83; 11.72 and 8.95% less in MCAO+Rad at 2, 14 and 28 days, respectively). MCAO caused a significant neurological deficit in both irradiated and non-irradiated animal groups compared to preischemia ($p<0.05$, ANOVA) but did not differ betweens groups. Surprisingly, an increase in the number of cells with DNA strand breaks detected in all animals (TUNEL), was one third less in MCAO+Rad group at 28 days after stroke.

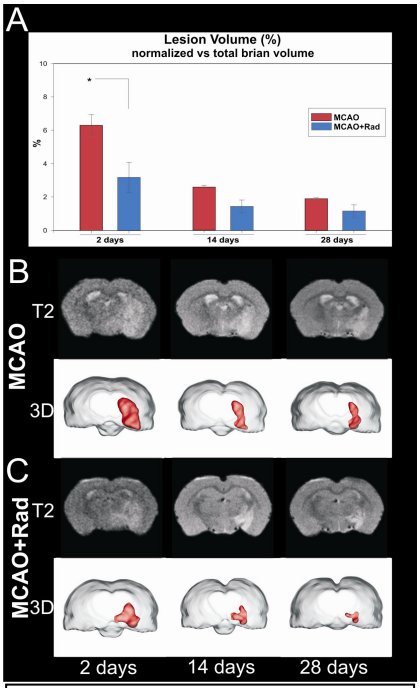
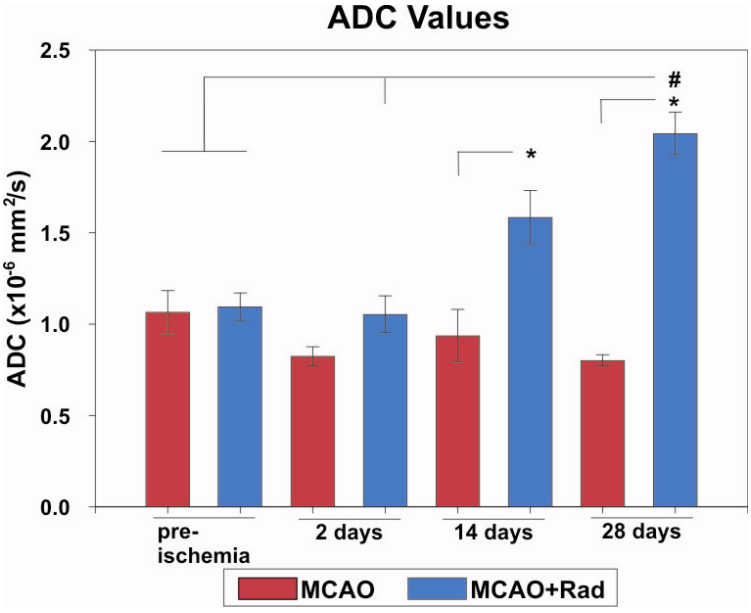


Figure 1. Neuroimaging and 3D reconstruction after MCAO induction at 2, 14 and 28 days. A. The lesion volume was markedly higher in MCAO group compared with MCAO+Rad at 2 days after ischemia induction (*$p<0.01$, ANOVA) and remained elevated for 28 days post injury. **B, C.** T2 and DWI 3D reconstructions show clearly a reduced lesion size in the MCAO+Rad compared to MCAO only.



Conclusion: Exposure to 8Gy at 10 days prior to MCAO resulted in a 2 fold increase in ADC that was not observed in MCAO only animals. Interestingly, lesion volumes were reduced in these animals. Thus, radiation exposure prior to stroke onset, appears to reduce cytotoxic edema but accentuates increased ADC values at later time points, with a tendency to decrease in apoptosis. Our results suggest that stroke outcomes in previously irradiated patients differ from the normal population and results in less brain edema in acute stages. Further studies are needed to understand long-term outcomes.

Figure 2. ADC evaluation at 2, 14, and 28 days. ADC values were noticeably increased at 14 and 28d points compared with preischemia ADCs with a 2-fold increase at 28 days in radiation exposed MCAO animals (#$p<0.001$, ANOVA). Differences between ADC values in MCAO and MCAO+Rad animals were significant at 14 and 28 days after ischemic brain injury (*$p<0.01$, ANOVA).