Longitudinal Anatomical and Diffusion MRI Evaluation in Rabbit External Capsules and Hippocampi after Cerebral Hemisphere Radiation Exposure

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Purpose

In the study, we used a linear accelerator as the source of radiation to establish an adult rabbit model for single-dose cerebral hemisphere exposure radiation-induced brain injury, and afterward, it longitudinally evaluated the changes in various brain compartments on a 1.5T clinical MR scanner by using T2 weighted imaging (T2WI) and diffusion tensor imaging (DTI) indices: fractional anisotropy (FA), mean diffusivity (MD), axial diffusivity (AD) and radial diffusivity (RD) mapping. It is hoped that this experimental model can be used to evaluate the neuro-toxic adverse effects of irradiation treatment.

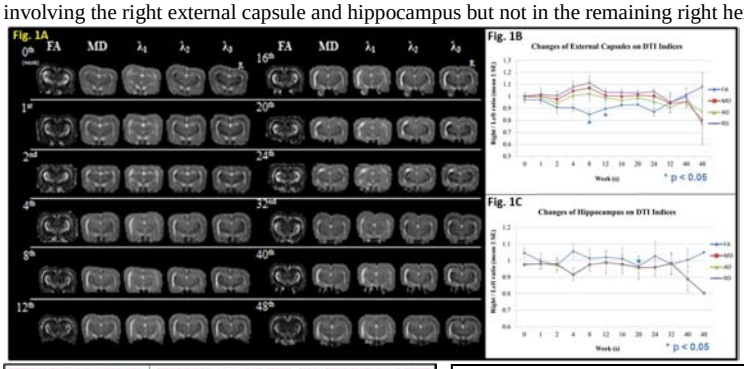
Materials and Methods

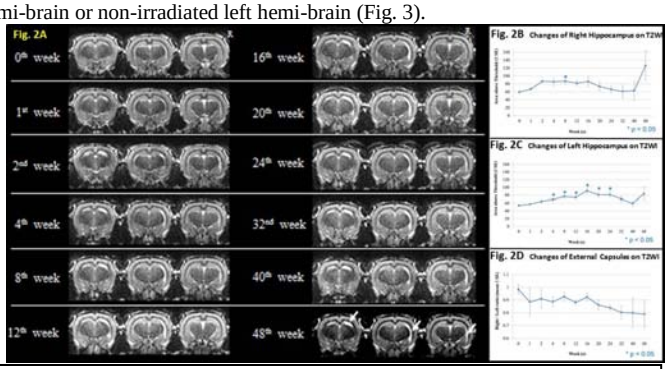
Five male New Zealand rabbits of one year of age received irradiation of 30 Gy (collimations = 4 cm x 5 cm) to the right hemi-brain with a single highly collimated 6MV photon beam from a Varian CL21EX linear accelerator (Varian, Palo Alto, CA, USA) under anesthesia. Brain MRI examinations were performed before irradiation and at 1st to 48th week post-irradiation (totally 12 time points) on a 1.5T MR scanner (Sonata, Siemens, Erlangen, Germany) with double loop array coils. Whole brain coronal T2WI were obtained using TR/TE = 4330/114 ms, resolution = 0.19 x 0.39 x 1.5 mm^3, number of slices = 30. DTI data were acquired using a multi-slice, single-shot spin echo EPI sequence with TR/TE=2900/128 ms, resolution = 0.78 x 0.78 x 2 mm^3, number of slices = 12 (contiguously from the genu of the corpus callosum to the end of the cerebrum). They were obtained using 12 diffusion-encoding directions with b values changing from 0 to 2,000 s/mm^2.

In data analysis, FA, MD, AD and RD maps were first calculated from DTI data using DSI studio (NTU, Taiwan) (Fig. 1A). Using imageJ (NIH, USA), ROIs were drawn manually on three consecutive slices of the DTI maps (both bilateral ECs and hippocampi) and the T2WI (only bilateral ECs). All the results were expressed as mean ± standard error (SE), and the ratios of right (injury) / left (control) were calculated for statistical analysis. Since the affected hippocampus was the only compartment which could be visually inspected as an increased signal intensity on T2WI (only occurred on the superior-lateral aspect, Fig. 2A), for a more appropriate specification of the change, we set the mean gray scale value of the control (left) side hippocampus as a threshold and then summarized the areas above the threshold of the three slices in each side of the hippocampus separately. All the results were calculated for statistical analysis. Paired t-test was used to detect statistical differences between the pre- and the post-irradiation time points. A p-value of <0.05 was considered to indicate statistical significance. After 48 week of MRI scans, rabbits were sacrificed for histopathological evaluation, and stained with hematoxylin and eosin (H&E) and Luxol fast blue (LFB).

Results

DTI indices: In EC, there was a continuing decrease followed by a gradual recovery in the FA R/L ratio during the follow-up time points, and the differences reached statistical significance at the 8th week (p-value = 0.0193) and the 12th week (p-value = 0.0373) post-irradiation. The gradual increase was followed by recovery in the RD and the MD R/L ratios and the AD R/L ratio showed a decrease at the 2nd week and the 32nd week post-irradiation (Fig. 1B). In hippocampus, the differences reached a statistical significance at the 20th week in the FA R/L ratio (p-value = 0.0405) and there was no clear trend in any DTI indices (Fig. 1C). *T2WI:* In hippocampus, the area above the threshold of the right hippocampus rapidly increased in the first two weeks and then reached a plateau (2nd to 16th week), and this was followed by a gradual recovery after the 16th week post-irradiation. The differences reached a statistical significance at the 8th week (p-value = 0.0341) post-irradiation (Fig. 2B). In left hippocampus, the area above the threshold showed a continuing, gradual increase in the acute to early delayed phases followed by a rapid recovery after the 24th week post-irradiation (vertex at the 16th week). The differences reached a statistical significance at the 4th to 24th week (p-value < 0.05) post-irradiation (Fig. 2C). In EC, there was a continuing, gradual decrease at all the follow-up time points (Fig. 2D). *Histopathology:* One rabbit showed significant radiation-related alterations in right hemi-brains in both the H&E and LFB sections. Large areas of confluent coagulation necrosis and loss of the myelin sheath involving the right external capsule and hippocampus but not in the remaining right hemi-brain or non-irradiated left hemi-brain (Fig. 3).





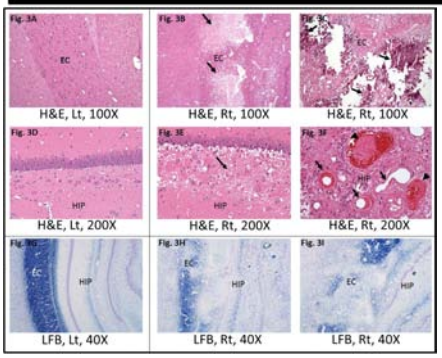


Fig. 1 (A) FA, MD, λ_1, λ_2, and λ_3 mapping were calculated from the DTI data at baseline and at the first to 48th week post-irradiation. **(B-C)** Longitudinal R/L ratio changes of DTI indices in the external capsule and hippocampus after irradiation.

Fig. 2 (A) T2WI in three consecutive slices at baseline and at the first to 48th week post-irradiation. **(B-D)** Longitudinal area changes (right and left hippocampus) and R/L ratio changes (external capsules) of T2WI after irradiation.

Fig. 3 Histopathological evaluations of injury (Rt) and control sides (Lt) of the rabbit after 48 weeks post-irradiation including H&E **(A to F)** and LFB **(G to I)** stain. **(A, D, G)** No significant radiation-related alterations in the left hemi-brain. **(B)** Confluent necrosis (arrow) in the right external capsule (EC). **(C)** Dystrophy calcifications (arrow) in the right EC. **(E)** Demyelination (arrow) in the right hippocampus (HIP). **(F)** Prominent hyalinization, fibrinoid deposition of vessel walls (arrow) with luminal thrombosis (arrow head) in the right HIP. **(H, I)** Decreased coloration in the right EC, indicating loss of the myelin sheath. Disorganization of myelin fibers in both the right EC and HIP.

Discussions

Changes of AD represent initially increasing and then gradually reducing the reactive astrogliosis in the early delayed phase and axonal degeneration in the late delayed phase. RD reflected transient demyelination and then remyelination. Decreased MD is the most sensitive measure for detecting cytotoxic edema from ischemic injury. Changes of FA represent poor tract integrity and can be explained by histopathological changes, including reactive astrogliosis, vasogenic edema and demyelination. Increasing signal intensity of T2WI only in the affected hippocampus (limited to the superior-lateral aspect) can be explained by the fact that relatively more radio-sensitive of the hippocampus, and the hippocampal cornu ammonis 1 (CA1), which is located in the superior-lateral aspect of the hippocampus, is even more vulnerable to the effects of radiation. An initial, continual, gradual increase in the long T2 area of the unirradiated left hippocampus can be explained by background radiation exposure.

Conclusions

We successfully established a rabbit model for the longitudinal evaluation of radiation-induced brain injury using a clinical MR scanner, and found that both DTI indices and T2WI have their own advantages in detecting post-irradiation changes in different brain compartments. These feasible working protocol and multiparametric MRI measurements can be valuable in monitoring the pathophysiological cascades by using clinical MR scanner during the course of radiation-induced brain injury and may shorten the distance from bench to bedside.

References [1] Wang S, et al. Cancer Res. 2009; 69(3): 1190-8. [2] Rola R, et al. Exp Neurol. 2004; 188, 316-330. [3] Shi L, et al. Radiat Res. 2006; 166, 892-899. [4] Obenaus A, et al. Radiat Res. 2008; 169, 149-161.