Quantitative multi-parametric assessment of a radiation-induced encephalodysplasia CNS model using magnetic resonance imaging


1Tohoku University, Sendai, Miyagi, Japan, 2National Institute of Radiological Sciences, Chiba, Chiba, Japan, 3Tsukuba International University, 4National Institute of Radiological Sciences, 5Singapore Bioimaging Consortium

INTRODUCTION
Radiological examinations, such as abdominal x-ray or CT, during pregnancy have been associated with a slightly increased risk of brain tumor [1]. Radiation exposure during the embryonic period causes various diseases such as hydrocephalus, microcephaly and other brain disorder [2]. Recently, it has been demonstrated that manganese-enhanced MRI (MEMRI), using a MnCl₂ contrast agent, can be used to visualize neuro-architecture [3]. MEMRI may also be useful in the evaluation of radiation-induced central nervous system (CNS) disorder. In this study, we non-invasively assessed developmental CNS disorder induced by prenatal x-ray exposure with quantitative MRI. Changes in longitudinal relaxation time (T1) induced by intracellular Mn²⁺ contrast agents were quantitatively observed in the CNS of normal and radiation-exposed rats. The apparent diffusion coefficient (ADC) and transverse relaxation time (T2) were also assessed. A histological study with Hematoxylin-Eosin (cell density and necrotic changing), Activated Caspase-3 (apoptotic changing), and Glial fibrillary acidic protein (astrogliosis) was also performed to evaluate tissue/cellular degeneration.

MATERIALS AND METHODS
Pregnant SD female rats (n = 4, 250–280 g, Japan SLC, Hamamatsu, Japan) were randomly divided into two groups. One group was exposed to a single dose of whole-body x-ray radiation (1.5 Gy) on day-15 of pregnancy. The X-ray irradiation conditions were 200kVp, 20mA, 0.5 mm Cu + 0.5 mm Al filter, 110cm distance from focus to object and 0.27–0.28 Gy/minute dose rate [4]. Two weeks after birth, 10 neonatal male rats were randomly picked from the normal (n = 5, 2WN group) and the radiation-exposed (n = 5, 2WR group) animals. MnCl₂ (0.002 ml/g, 50 mM, Sigma-Aldrich) was slowly infused 24 hours before MRI acquisition. Coronal multi-slice T₁-weighted MR images (multi slice SE, TR/TE = 400/9.57 ms, slice thickness = 1.0 mm, matrix = 256×256, field of view = 25.6 × 25.6 mm², average = 4), T₁-mapping (2D Look-Locker, TR/TE = 10000/10 ms, slice thickness = 1.0 mm, matrix = 64×32, field of view = 25.6×12.8 mm², average = 1), diffusion imaging and T₂-mapping were acquired using a 7.0 T-MRI (Magnet: Kobelco + JASTEC, Japan. Console: Bruker Biospin, Germany) in combination with a volume coil for transmission (Bruker) and 2ch phased array coil for reception (Rapid Biomedical, Germany). T₁-mapping was performed using a multislice SE sequence (slice thickness = 1 mm; FOV = 25.6 × 25.6 mm²; matrix = 128 × 128; number of repetitions = 1; TR = 3000 ms) with echo-times ranging from 10 to 140 ms in steps of 10ms. Diffusion imaging was performed using multi-slice echo planar imaging (slice thickness = 1 mm; field of view = 25.6 × 25.6 mm²; matrix = 128 × 128; number of repetitions = 1; TR = 3000 ms; TE = 33 ms). ADC maps were calculated from diffusion-weighted images acquired at six b-values (b = 0, 250, 500, 750, 1200, 1500 s/mm², δ = 3 ms, Δ = 23 ms). After the MRI experiments, all rats were prepared under anesthesia for histology using paraformaldehyde perfusion. To evaluate T₁, a cortical region-of-interest (ROI) was defined on the MEMRI at a position approximately -1.0 mm posterior to the Bregma. Statistical analysis was performed using 2-way ANOVA and an unpaired t-test (**P<0.01, ***P<0.001).

RESULTS and DISCUSSION

(A) Cortical T₁ for the 2WN animals was lower than in other brain areas such as the thalamus and amygdala. In (B), cortical T₁ is similar to that in other brain areas such as thalamus and amygdala. In (C), cortical T₁ is compared for the 2WN and 2WR groups (p < 0.05). ADC maps for the (D) 2WN and (E) 2WR groups are also shown. In (D), the cortical ADC values are lower than in other brain areas such as the thalamus and amygdala. In (E), the cortical ADC values are similar to those in other brain areas. In (F), the cortical ADCs for 2WN and 2WR were 0.82 ± 0.05 and 1.03 ± 0.03 mm²/s, respectively. The ADC for 2WR was significantly higher than for 2WN (p < 0.001).

Figure 1: Typical T₁WI. Panels A, B, C and D: MEMRI of normal rat brain. Panels E, F and H: MEMRI of radiation-exposed rat. Red arrows indicate ventricle dilatation.

Figure 2: T₁-mapping. Typical coronal T₁-maps. (A) T₁-mapping prior to MnCl₂ administration for normal rat. (B) T₁-mapping prior to MnCl₂ administration for radiation-exposed rat. (D) T₁-mapping after MnCl₂ administration for normal rat. (E) T₁-mapping after MnCl₂ administration for radiation-exposed rat.

Figure 3: T₁-mapping. (A) Cortical T₁ values after MnCl₂ administration. (B) The ratio of cortical T₁ before and after MnCl₂ administration for radiation-exposed rat was significantly lower than that for normal rat (p < 0.001). This result suggests that the uptake of MnCl₂ in cortex for radiation-exposed rat was lower than that for normal rat cortex. Figure 4: T₂ and ADC maps. Typical T₂ and ADC maps for the 2WN and 2WR groups are presented. (A) T₂-mapping for the 2WN animals was lower than in other brain areas such as the thalamus and amygdala. In (B), cortical T₂ is similar to that in other brain areas such as thalamus and amygdala. In (C), cortical T₂ is compared for the 2WN and 2WR groups (p < 0.05). ADC maps for the (D) 2WN and (E) 2WR groups are also shown. In (D), the cortical ADC values are lower than in other brain areas such as the thalamus and amygdala. In (E), the cortical ADC values are similar to those in other brain areas. In (F), the cortical ADCs for 2WN and 2WR were 0.82 ± 0.05 and 1.03 ± 0.03 mm²/s, respectively. The ADC for 2WR was significantly higher than for 2WN (p < 0.001).

Figure 5: HE, Activated Caspase-3 and GFAP histology. The top row (A, B) presents HE stained histological slices of the cortex for each group. Cell-density in the radiation-exposed cortex (2WN) was significantly lower than in normal rat cortex (C, p < 0.001). The middle row (D, E) contains Activated Caspase-3 stained histological slices of the cortex. Many activated-Caspase-3-positive cells (an example is indicated by the arrow), were observed in the cortex for the 2WR group. The density of Activated Caspase-3 for the radiation-exposed group increased significantly (F, p < 0.001). The bottom row (G, H) shows GFAP-stained histological slices in the cortex. The GFAP-positive area in the radiation-exposed rats was larger than for the normal animals (I, p < 0.001).

CONCLUSION
We found that: 1) decreased MnCl₂ uptake for radiation-exposed rats (as indicated by longer MEMRI T₁) (Fig. 3)) and increase of activated-Caspase-3-positive cells (Fig. 5F) imply a decrease of cell viability (apoptotic cytotoxicity) after prenatal radiation exposure; 2) larger T₁ and ADC (Fig. 4) and reduction in the number of HE-positive cells (Fig. 5C) implies a decrease in CNS cell density after radiation exposure. Proliferation of astroglia was also observed (Fig. 5I). Kawai et al recently reported that proliferation of astroglia is the predominant mechanism behind MEMRI enhancement after focal brain ischemia [5]. In our prenatal radiation-exposure model, both proliferation of astroglia and decrease of cell density were observed (Fig. 5C, I). Therefore, we hypothesize that the decrease in cell number counteracts the proliferation of astroglia in the radiation-exposure model. In conclusion, quantitative T₁ mapping with MEMRI in combination with ADC and T₂ mapping provides comprehensive multiparametric information for the evaluation and diagnosis of prenatal radiation exposure.

ACKNOWLEDGEMENT
The authors would like to thank Jeff Kershaw, Sayaka Shihuta and Takeo Shimomura for valuable assistance.