Quantitative assessment of prenatal X-ray exposure using longitudinal gadolinium-, manganese-enhanced and perfusion MRI in neonatal rats

S. Saito[1], K. Sawada[2], X-Z. Sun[3], T. Suhara[4], I. Kanno[4], and I. Aoki[4]

[1]Department of Molecular and Neuroimaging, Graduate School of Medicine, Tohoku University, Sendai, Miyagi, Japan, [2]Laboratory of Anatomy, Department of Physical Therapy, Faculty of Medical and Health Sciences, Japan, [3]Regulation Science Research Group, National Institute of Radiological Sciences, Chiba, Japan, [4]Molecular Imaging Center, National Institute of Radiological Sciences, Chiba, Japan

INTRODUCTION It is known that a high dose of ionizing radiation (15–20 Gy) causes cellular heterogeneity, spinal cord myelopathy, white matter necrosis (leukoencephalopathy), and microvascular damage [1-4] in the CNS. Microvascular damage causes increased endothelial cell swelling, vascular permeability and edema, lymphocyte adhesion and infiltration, and apoptosis [1,2] in the acute phase. In particular, 20% of the apoptotic cells observed after a mild dose of radiation were endothelial cells in the CNS [5]. In the final phase, the microcirculation disorder and ischemic responses such as capillary collapse, teleangiectasias, and scarring and fibrosis are observed [1,2]. Therefore, recovery of 'brain perfusion' should be a primary target in the treatment of radiation injury.

The embryo is sensitive to radiation. For example, irradiation by as little as 0.1 Gy is lethal for a fertilized egg, while milder radiation exposure of the embryo can cause various diseases such as hydrocephalus and microcephaly. Therefore, *in vivo* evaluation of radiation damage in the CNS is important for the treatment of radiation injury. Recently, a rapid, quantitative T_1 mapping method has been established using Look-Locker acquisition [6]. This T_1-mapping method allows quantitative evaluation of the dynamics of T_1 contrast agents in the CNS. Gd-DTPA is a representative intravascular and extracellular T_1 agent in the intact CNS. When the BBB is broken, the Gd-DTPA can leak from the capillaries but is unable to enter cells. On the other hand, Mn^{2+} T_1 contrast agents can enter cells through Ca^{2+} channels and remain for a long time after BBB disruption. In addition, Mn^{2+} may reflect brain damage such as necrotic Ca^{2+} influx, Mossy fiber sprouting in the hippocampus, and/or astro-gliosis [7-9]. Therefore, tissue characterizations using contrast agents with different properties provides additional cellular and molecular information for radiation research and treatment. Our goal is to investigate differences in the longitudinal dynamics between normal and irradiated subjects using both Gd-DTPA and Mn^{2+} T_1 agents. In addition, the contribution of the BBB permeability was tested in 1-week neonatal and 2-week rats [10]. Brain perfusion was assessed using a quantitative spin-labeling method [11], and the endothelial/BBB condition evaluated with immunohistological staining.

MATERIALS AND METHODS Male SD rats were arranged into four groups: (1) 1-week neonatal normal rats (1WN, n =5), (2) 2-week rats (2WN, n =5), (3) 1-week neonatal radiation exposed rats (1WR, n =5), and (4) 2-week radiation injury model rats (2WR, n =5). Two MRI contrast agents were used for every animal: (1) Gd-DTPA for the assessment of BBB formation and vessel damage, and (2) $MnCl_2$ for assessment of neuronal activity or deletion. First of all, pregnant SD female rats (n = 4, 250~280 g. Japan SLC, Hamamatsu, Japan) underwent a single exposure to whole-body X-ray irradiation at a dose of 1.5 Gy on day 15 of pregnancy. 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 [12]. After birth, we used 26 neonatal male rats selected at random including normal (n =10) and radiation exposed rats (n =10). The controls included 1WN (n = 5) and 2WN (n = 5) rats, and the radiation exposed included 1WR (n = 5) and 2WR (n = 5) rats. In addition, 6 rats (3 normal and 3 radiation exposed) were used for perfusion evaluation. Figure 1 shows the experimental protocol. The Gd-DTPA (Magnevist, 0.04ml/g, 50mM, Bayer) and MnCl2 (0.04ml/g, 10mM, Sigma-Aldrich) were injected during continuous and repetitive acquisitions for T_1-mapping. Coronal multi-slice T_1-weighted MR images (T1WI; Multi slice SE, TR/TE = 400/9.57 ms, slice thickness = 1.0 mm, matrix = 256*256, field of view = 25.6 *25.6 mm, average = 4) and 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 mm, average=1) were obtained using a 7.0 T-MRI (Magnet: Kobelco +JASTEC Japan; Console: Bruker Biospin, Germany) with a volume coil for transmission (Bruker) and 2ch phased array coil for receiving (Rapid Biomedical, Germany). Perfusion imaging was performed using an arterial spin- labeling technique (EPI-FAIR) with T_1-mapping (slice thickness = 2 mm; FOV = 2.24*2.24 cm; matrix = 128*64; number of repetitions = 2; TR = 18000 ms; TE = 26 ms). All rats were euthanized and perfusion fixation was performed for histology after the MRI data acquisition. To evaluate changes in T_1, the region of interest (ROI) was defined as the entire cortex. T_1 values were normalized using the first image of T_1 mapping without a contrast agent. Two-way ANOVA was used for statistical comparisons (Prism, Version 5, USA).

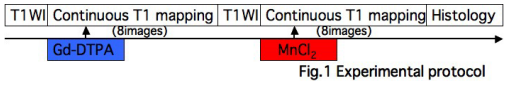

Fig.1 Experimental protocol

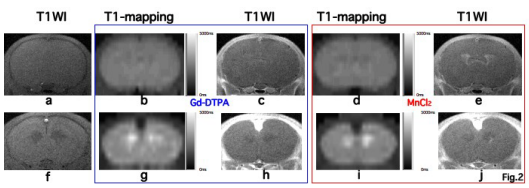

Fig.2

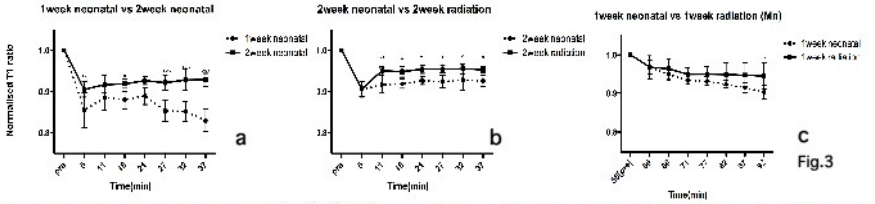

Fig.3

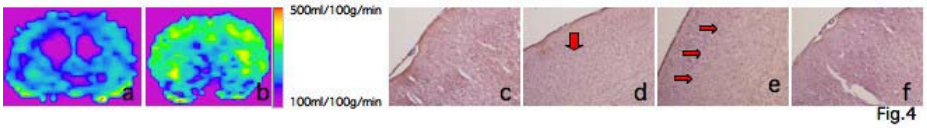

Fig.4

RESULTS Figure 2: Typical coronal T1WI and T_1-mapping of a 2-week normal rat (a~e) and a 2-week radiation exposed rat (f~j) (a), (f) T1WI without Gd-DTPA (b), (g) T_1 mapping 10 minutes after the Gd-DTPA injection (c) ,(h) T1WI 45 minutes after the Gd-DTPA administration (d), (i) T_1 mapping at 10 minutes after MnCl2 administration (e), (j) T1WI 45 minutes after MnCl2 administration. Figure 3: Longitudinal observation of the normalized T_1 ratio in the cortex. (a) Normalized T1 ratio in normal 2WN was significantly higher than the 1WN at 27 min, 32 min, and 37 minutes after Gd-DTPA injection. (b) Normalized T1 ratio in the 2WR was significantly higher than the 2WN 11- minutes after Gd-DTPA injection. (c) Normalized T1 ratio in 1WR was significantly higher than the 1WN at 37 minutes after MnCl2 injection (*:P<0.05, **:P<0.01, ***:P<0.001). Figure 4: Typical perfusion mapping using EPI-FAIR in the (a) 2WR and (b) 2WN. Radiation exposure led to a decrease in CBF in comparison with the normal rat brain (a) 110.3 ± 15.4 ml/100g/min, (b) 205.7 ± 31.3 ml/100g/min ($P < 0.01$, paired t-test). Fig. 4c and 4d show laminin staining at x40 magnification demonstrating the endothelial cell structure. In the 2WR (d), laminin staining indicated disruption of vascular endothelial cells (disappearance of blood vessels). Fig. 4e and 4f show albumin staining. Albumin exists only in the intravascular region in the intact BBB animal. Albumin leaked into the extravascular space in the CNS (Fig. 4 right side from arrows).

DISCUSSION 1) It was confirmed using Gd-DTPA that the BBB permeability of a normal new born (1 week) is higher than a normal at 2 weeks. Albumin staining also supported this result. 2) The normalized T_1 ratio in normal (2-weeks) was smaller than the irradiated (2-weeks) subjects after Gd-DTPA injection. Luminin staining also suggested vascular endothelial cell damage in the irradiated model (2WR). 3) Mn accumulation in the irradiated brain (1WR) was smaller than the normal (1WN). This suggests that the number or density of intact neural cells decreased due to the irradiation. Gd-DTPA and perfusion MRI is a useful technique for BBB and microcirculation assessment [13] in subjects exposed to ionizing radiation. In addition, Mn^{2+} contrast agents used in conjunction with quantitative T1 mapping can be used as a cellular density/activity indicator for a neonatal animal.

REFERENCE [1] Radiation tolerance of normal tissues. Front Radiat Ther Oncol 1989;23:1-443.[2] Baker DG, Cancer Invest 1989; 7:287-294.[3] Pena LA.Cancer Res 2000; 60:321-327 [4] Butt M. J Physiol(Lond) 1990. [5] Louis. J Neurosci Res1992; 31:193-204.[6] Chuang KH. Magn Reson Med 2006; 55:604-611. [7] Kawai et al, ISMRM 2007:1215 [8] I.Aoki, Neuroimage 22 (2004) pp.1046–1059 [9] ImmonenRJ.Neuroimage 2008; 40:1718-1730 [10] Janeczko K. Brain Res1997; 770:237-241. [11] KimSG. Magn Reson Med. 1997 Mar;37(3):425-35. [12] Sun Xue-Zhi. Journal of Radiation Research. 2002 [13]Grillon E. NMRBiomed2008; 21:427-436.

ACKNOWLEDGEMENTS The authors would like to thank Mr Nick Dover, Ms S. Shibata, Mr. T. Shimomura and Mr Jeff Kershaw for their assistance.