

Evaluation of hippocampal degeneration after prenatal radiation exposure using Manganese-enhanced MRI (MEMRI)

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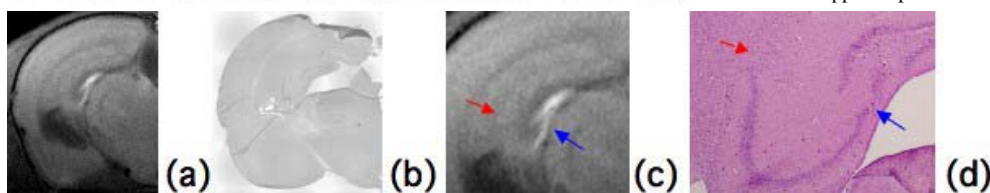
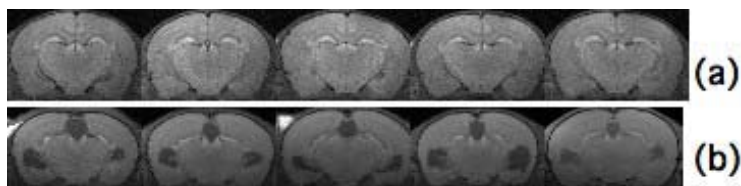
INTRODUCTION

Radiation exposure during the prenatal period causes various diseases such as hydrocephalus. It is known that each brain area has its own tolerance to radiation. It has also been shown that radiation exposure-induced hydrocephalus is responsible for dilatation of the cerebral ventricles and disruption of vascular endothelial cells [1]. In addition, histological examination suggested that radiation-exposure also induces hippocampal atrophy [2]. Recent developments with manganese-enhanced MRI (MEMRI) have shown that it can clearly distinguish the hippocampal formation [3] and cortical layers [4] in-vivo. There are several hypotheses for the mechanism of manganese enhancement in brain disorders such as astrogliosis [5], microglia migration [6], mossy-fiber sprouting [7], and Mn-SOD expression [8]. Our goal was to evaluate the change in volume of the manganese-enhanced hippocampal area, hippocampus and entire brain of the rat with or without X-ray exposure during the prenatal period. Histology in the degenerated hippocampal area is also evaluated.

MATERIALS AND METHODS

Pregnant SD female rats (n = 4, 250~280 g, Japan SLC, Hamamatsu, Japan) were used. Two rats received a single exposure to whole-body X-ray irradiation at a dose of 1.5 Gy on day 15 of pregnancy, while the other two were used as controls. 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 [2]. Four weeks after birth, MRI scans were performed on the normal control (N=5) and radiation-exposed (n = 5) progeny. Coronal 3D T₁-weighted MR images (TR/TE = 400/9.57 ms, matrix = 256*224*45, field of view = 25.6 mm*22.4 mm*9.0 mm, average = 4, spatial resolution = 100*100*200 μm) 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 a 2-ch phased array coil for receiving (Rapid Biomedical, Germany). Osmotic pressure controlled MnCl₂ (0.02ml/g, Sigma-Aldrich) was given by infusing 75 ml/kg of a 50 mM MnCl₂ solution at a rate of 0.4 ml/hour through the tail vein 24 hours before MRI scanning. ROIs encompassing the right hippocampus and whole brain area were manually selected by a trained observer. The hippocampus slice corresponded to a level approximately -2.9 mm posterior from the Bregma. The enhanced hippocampus lesion was defined by thresholding at mean+2SD of the signal intensity of a ROI in the caudate putamen. Statistical calculations were performed using Excel add-in tools.

RESULTS



arrows)

Fig.1 (a) Normal control rat: typical 3D T₁-weighted image 24 hours after MnCl₂ administration (4 weeks old, n=5) (b) Radiation-exposed rat: typical 3D T₁-weighted image 24 hours after MnCl₂ administration (4 weeks old, n=5) Manganese-enhanced MRI can clearly distinguish the hippocampal formation, CA1, CA2 and CA3 in the intact rats. In contrast, CA1 was not enhanced in the radiation-exposed rats. The CA2, CA3, and DG were strongly enhanced by MnCl₂, although deformation of the hippocampal formation was observed.

Fig.2 (a) 3D MEMRI, (b) HE staining, (c) magnification of 3D MEMRI, and (d) magnification of HE staining in a radiation-exposed rat (4 weeks old, right hemisphere). A CA1 pyramidal-cell layer disappeared for radiation injured model. (Red arrows in Fig2 (c) and (d)) In addition, degeneration of CA3 and dentate gyrus was observed. (Blue

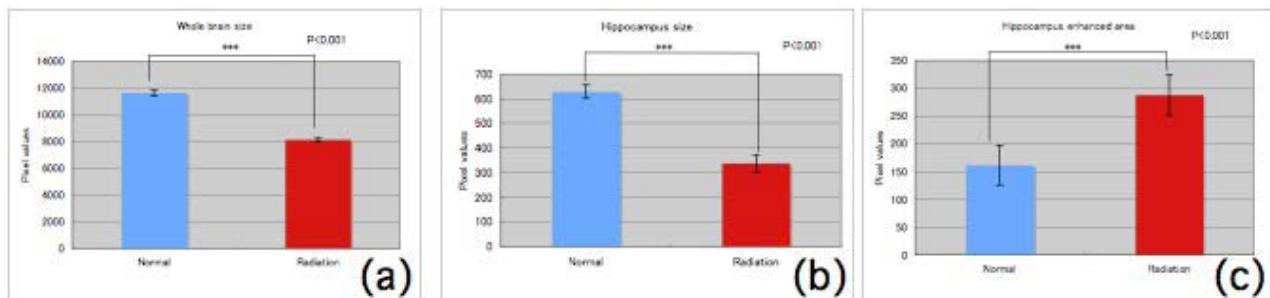


Fig3. Comparison of (a) whole brain volume, (b) Hippocampal area, and (c) manganese-enhanced area between the normal and radiation-exposed model as determined from MEMRI. Brain volume and hippocampal area of the radiation-exposed model were significantly decreased (P<0.001, unpaired t-test). Manganese-enhanced area of the radiation-exposed model was significantly increased (P<0.001, unpaired t-test).

DISCUSSION and CONCLUSION

X ray irradiation during the prenatal period showed a remarkable degeneration in brain formation, especially in the hippocampus. Our study demonstrated: 1) Atrophy of the whole brain with ventricular dilatation was observed in the radiation-exposed model (Fig. 1); 2) The atrophy of hippocampus was confirmed by MEMRI and HE staining (Fig 3b and Fig. 2c) in the radiation exposure model in the rat; 3) The size of the manganese-enhanced area in the radiation-exposed rats increased in the hippocampus (Fig. 3c). It is speculated that the increase in manganese-enhanced hippocampal area for the radiation-exposed model was caused by (1) mossy fiber sprouting and/or (2) astro-gliosis. MEMRI is a useful tool for evaluating brain degeneration induced by radiation exposure.

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