学 位 論 文 の 内 容 の 要 旨

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X-irradiation induces acute cognitive decline via transient synaptic dysfunction

【Background and Aim】

Radiation therapy is often used for the treatment of brain tumors; however, radiation-induced brain injury mostly observed and symptomatically found chronically after radiation therapy, and the main underlying cause is thought to be cell death. Radiation-induced brain injury is classified as acute, early delayed and late delayed injury. Early delayed injuries start to occur more than one month after irradiation, and late delayed injuries occur after one year or longer. The acute effects of irradiation are well known and it has been reported that the acute injuries manifest within days of exposure. However, very few studies have investigated the underlying mechanisms of the acute effects of irradiation. Because mature neurons are relatively radiation-resistant, the acute effects of injury might be associated with synaptic dysfunction. In this study, we have been focusing on the acute effects of X-irradiation and possibility of its underlying mechanism.

【Methods】

Ten- to 11-week-old male mice were used (C57BL/6N; CLEA Japan, Inc., Japan; SLC Co., Ltd., Shizuoka, Japan). X-rays were generated using a Shimadzu X-TITAN 225S X-ray generator (Shimadzu Corporation, Kyoto, Japan), and a dose rate of 1.3 Gy/min was used to irradiate mice. The mice were anesthetized with an intraperitoneal injection of tribromoethanol (Avertin; 250 mg/kg; Sigma-Aldrich, St. Louis, MO), placed in a stereotaxic frame (Narishige, Tokyo, Japan), and irradiated. We used 10-13 mice per group for behavioral analysis, 3-4 mice (4-7 slices from one animal) per group for immunohistochemical analysis and three mice per group for western blot analysis.

Fear memory conditioning was performed at 30 minutes before the whole brain irradiation, 7 hours and 24 hours after the whole brain irradiation. The mice were subjected to memory tests 24 h after the conditioning, and context-dependent fear memory was examined.

For the immunohistochemical and western blot analysis of the mouse brain, the left hemisphere was exposed to the radiation. The right hemisphere and the remainder of the body of the mouse were covered with a lead plate (1 cm thick) to protect from radiation exposure.

For the immunohistochemical analysis 30-μm-thick coronal sections of the brain were cut [−3.64 mm to −1.24 mm from the bregma, using a mouse brain atlas as reference] using a cryostat (CM3000, Leica, Wetzlar, Germany). The sections were incubated overnight with the primary antibody at 4 °C, then incubated with the secondary antibody for 1 h, rinsed with PBS, and then mounted. The sections were analyzed using a confocal laser scanning microscope (Fluoview FV1000, Olympus, Tokyo, Japan). The intensities of drebrin and synapsin I were analyzed from four random regions of interest (ROI) in the molecular layer of the dentate gyrus (DG) and analyze the ratios of the immunointensity of irradiation side / non-irradiation side. The

number of positive doublecortin (DCX) cells was quantified in the granular cell layer of DG.

For the western blot analysis; after eight hours of irradiation, irradiated and non-irradiated sides of hippocampal homogenates of 10-week old sham and irradiated mice were prepared. Aliquots of whole homogenates were equalized to 240 μg wet weight of hippocampal tissue/lane were subjected to SDS-PAGE and transferred to polyvinylidene fluoride membrane. The membrane was incubated with appropriate primary and secondary antibodies. Peroxidase activity was detected using chemiluminescence reagents (Immobilon Western Chemiluminescent HRP Substrate, Merck Millipore, Billerica, MA), and then visualized with an image analyzer (LAS-3000, Fujifilm, Tokyo, Japan). Images were quantified with NIH ImageJ software.

【Results】

First we examined irradiation effect on fear memory formation using behavioral study. When mice were trained 30 min before irradiation and trained 7 h after irradiation, they showed a significantly lower freezing rate compared with the sham group in the contextual test suggesting that their memory formation was impaired. In contrast, when mice were trained 24 h after irradiation, they did not show any difference from the sham group. Thus brain irradiation induces a transient deficit in fear memory formation.

We then immunohistochemically analyzed the immunofluorescence intensity of the synaptic marker proteins drebrin and synapsin I. Also we analyzed the irradiation effect on newly generated neurons by quantitating the number of doublecortin-positive cells. The drebrin immunointensity ratio (irradiation side immunointensity / non-irradiation side immunointensity) in the molecular layer of the dentate gyrus significantly decreased 2 and 8 h after irradiation and returned to pre-irradiation levels 24 h following exposure. In comparison, there was no change in the synapsin I ratio. We found that the number of doublecortin-positive cells did not change 2 h after the irradiation, but was significantly reduced in the irradiated hemisphere compared with the control hemisphere 8 and 24 h after the exposure. We further quantified drebrin, synapsin I and β-actin protein content in X-irradiated hippocampi by western blotting and found no change in 8 h following the exposure of X-irradiation.

X- irradiation of the brain has acute memory impairment within 24 hours after exposure. The time course of the transient memory impairment was similar to that of transient drebrin loss from dendritic spines, suggesting that a perturbation of the neuronal network caused by massive simultaneous synaptic dysfunction underlies the X-irradiation-induced acute memory decline.

【Discussions】

In this study, immediate memory impairment induced by X- irradiation persisted for at least seven hours and back to normal within 24 hours. This transient memory impairment is associated with the transient decrease of drebrin, a post-synaptic marker. The transient decrease of drebrin is due to distribution change from the synapses because we found no change in drebrin protein content. On the other hand, there is no change of synapsin I, a pre-synaptic marker, within 24 h after exposure in this study. It is known that the changes in the presynaptic sites occur 1 week after exposure, therefore it seems that changes in presynaptic sites take more time and this suggests that presynaptic sites are more stable than postsynaptic sites. Interestingly, the density of the newly generated neurons which is known to play a role in fear memory formation was decreased significantly after 24 h while the memory function was intact. This suggests that an acute transient memory decline which occurs within 24 hours is due to synaptic dysfunction but not due to cell death of newly generated neurons.