学 位 論 文 の 要 旨

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学 位 論 文 名　Increased Vulnerability of Hippocampal CA1 Neurons to Hypoperfusion in Ataxia and Male Sterility (AMS) Mouse

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論 文 内 容 の 要 旨

INTRODUCTION

The ataxia and male sterility (AMS) mouse is a spontaneous autosomal recessive mouse variant derived from MRL/*lpr* strain. In the mouse, the ataxic gait is due to spontaneous degeneration of Purkinje cells in the cerebellum. The similar phenotype is demonstrated in Purkinje cell degeneration (*pcd*) mice, where *nna1* mutation was considered to be the reason. Indeed, a point mutation of *nna1* gene in AMS was proved.

The expression of nna1 in brain is detected in cerebellum, some subcortical areas, and hippocampus. The *nna1* mutation lead to degeneration of Purkinje cells in the cerebellum, but no cell degeneration in the hippocampus of *pcd* mice.

Although the roles of nna1 on spontaneous neuronal cell degeneration and regeneration after injury have been studied, its role in stress-induced neuronal cell death has not been studied. We hypothesized that AMS hippocampal CA1 neurons might be vulnerable to hypoperfusion-induced stress. The effects of hypoperfusion on neuronal degeneration in the hippocampal CA1 region and the related underlying mechanisms were investigated in AMS and WT mice with an established hypoperfusion model by transient bilateral common carotid artery occlusion (BCCAO).

MATERIALS AND METHODS

The *ams* mutation has been transferred to and maintained in the MRL/+ mouse, a mother strain of the MRL/*lpr*. The MRL/+ mice were used as the WT counterpart of AMS in this study. AMS homozygous mice were produced by mating heterozygous parents. Eight-week-old male WT and AMS mice were subjected to BCCAO for 10 min and sacrificed 1, 3, 7 and 28 days after BCCAO. The morphological analysis of hippocampal neurons was done by Nissl staining. Cellular apoptosis was monitored by terminal deoxynucleotidyl transferase dUTP-biotin nick-end-labeling (TUNEL) staining. The activation of apoptosis-related protein, glial cells accumulation, and oxidative DNA damages were analyzed by immunostaining of active Bax protein, ionized calcium binding adaptor molecule 1(Iba1, a microglial marker), glial fibrillary acidic protein (GFAP, an astrocyte marker), and 8-hydroxy-2'-deoxyguanosine (8-OHdG, an oxidative DNA damage marker), respectively. The anti-oxidative status in the hippocampus was monitored by glutathione assay. The mRNA expression of antiapoptotic factor of Bcl-2, proapoptotic factor of Bax, mitochondrial uncoupling protein 2 (UCP2), and nna1 in the hippocampus was checked by Real Time PCR. The protein expression of Bcl-2, Bax and nna1 in the hippocampus was investigated by Western blotting, respectively.

RESULTS AND DISCUSSION

The morphological analysis of hippocampal neurons showed no significant change up to 28 d after BCCAO in WT mice, but in AMS mice, many neurons in the CA1 region at 7 d and 28 d exhibited pyknotic nuclei or disappearance of Nissl substance. The CA1 neuronal number started to decrease significantly from 7 d and continued up to 28 d after BCCAO ($p < 0.05$). TUNEL-positive cells were rarely found in WT sham or BCCAO mice up to 28 d. However in AMS, the number of them was significantly increased at 7 d after BCCAO ($p < 0.05$). These results indicated that the delayed neuronal death in the CA1 region after hypoperfusion in AMS is due to apoptosis.

In WT, *Bcl-2* mRNA and protein were increased in hippocampus after BCCAO ($p < 0.05$), whereas those were not increased in AMS after BCCAO. Moreover, *Bax* mRNA was transiently increased at 1 d in AMS ($p < 0.05$). Only a few active Bax-positive cells were detectable in WT after BCCAO, but the significant increase in number was found in AMS mice ($p < 0.05$). The remarkable activation of Bax indicated the involvement of the mitochondrial apoptotic pathway in AMS.

The response of microglia and astrocytes to ischemia might play a role in ischemia-induced delayed neuronal death in the CA1 region. The activated morphology and increased number of microglia and astrocytes were displayed in both AMS and WT after BCCAO. Nevertheless, the microglial and astrocytic responses were not different between the WT and AMS, which implying that CA1 neuronal death in AMS might not be due to the response of glial cells.

Previous reports suggested that neuronal vulnerability to ischemia in the CA1 region might be due to excessive oxidative stress. We evaluated the BCCAO-induced oxidative stress by immunohistochemistry of 8-OHdG, an oxidative DNA damage marker. The 8-OHdG-positive cell number was increased significantly at 1 d after BCCAO in both WT and AMS mice ($p < 0.05$). At 3 d, the number of positive cells had decreased in WT mice, but it remained high in AMS mice ($p < 0.05$).

Next we evaluated the antioxidative status in WT and AMS. GSH, an antioxidant, was significantly increased in WT mice at 3 d after BCCAO ($p < 0.05$), whereas there was no increase in AMS mice up to 7 d after BCCAO. The mRNA level of UCP2, a regulator of oxidative stress, was upregulated at 1 d after BCCAO in WT mice ($p < 0.05$), but it was not increased in AMS mice after BCCAO. As GSH and UCP2 target in reducing oxidative stress through scavenging reactive oxygen species and uncoupling of oxidative phosphorylation, respectively, their failure of response to oxidative stress might lead to continuation of the oxidative DNA damage condition, and ultimately lead to apoptotic neuronal death in AMS.

The *nna1* mRNA levels in sham, 1 and 3 d after BCCAO were similarly detected in both AMS and WT. But nna1 protein was not expressed in hippocampus of AMS mice at any time point although it was expressed in hippocampus of WT mice. A recent report demonstrated the localization of nna1 in mitochondria where it plays an important role in bioenergetics. The absence of nna1 may link to altered bioenergetic functions of mitochondria, which might be the reason of no induction of GSH in AMS, as GSH synthesis requires enzymatic steps involving ATP.

CONCLUSION

The hypoperfusion induced oxidative DNA damage was continued in the CA1 neurons in AMS, which possibly caused by the failure of induction of GSH and UCP2. The increased vulnerability of the CA1 neurons to hypoperfusion in AMS possibly resulted from the continuation of the oxidative DNA damage.