学 位 論 文 の 要 旨

氏名 Liaury Kristian

学 位 論 文 名　Morphological Features of Microglial Cells in the Hippocampal Dentate Gyrus of Gunn Rat: a Possible Schizophrenia Animal Model

発 表 雑 誌 名　Journal of Neuroinflammation
（巻，初頁～終頁，年）　9:56, 2012

著 者 名　Kristian Liaury, Tsuyoshi Miyaoka, Toshiko Tsumori, Motohide Furuya, Rei Wake, Masa Ieda, Keiko Tsuchie, Michiyo Taki, Kotomi Ishihara, Andi Jayalangkara Tanra, and Jun Horiguchi

論 文 内 容 の 要 旨

INTRODUCTION

Schizophrenia is a complex and debilitating mental disorder with a prevalence of approximately 1% worldwide. Schizophrenia is still a major challenge in psychiatry, in part because the exact etiology remains unknown.

An accumulating body of evidence point to the significance of neuroinflammation and immunogenetics in schizophrenia, characterized by an increased serum concentration of several pro-inflammatory cytokines. Interestingly, microglial activation or increased microglial cellular density has also been suggested by postmortem studies, at least in subpopulations of individuals with schizophrenia. Moreover, a pro-inflammatory immune state influences the glutamatergic neurotransmission indirectly by bits effects on the tryptophan/kynurenine metabolism.

Previous studies showed a link between hyperbilirubinemia and schizophrenia. Schizophrenia patients have a significantly higher frequency of hyperbilirubinemia relative to patients with other psychiatric disorders and the general healthy population. Based on these facts, we propose that hyperbilirubinemia may play a role in the pathophysiology of schizophrenia.

Our previous study showed behavioral abnormalities, deficits in prepulse inhibition (PPI), and neuropathological changes in Gunn rats that are similar to the characteristics of schizophrenia. The Gunn rat, a mutant of the Wistar strain, has a genetic deficiency in glucuronyl transferase and has been used as an animal model of bilirubin encephalopathy. We found that a high serum

UCB concentration has a pathogenic effect on development of the brain and concluded that the Gunn rat may be used as an animal model of schizophrenia.

UCB in the CNS is toxic to neurons and associated microglia, the resident immune cells in the CNS. However, the effects of UCB on microglia in Gunn rats have never been investigated. Therefore, in the present study, we sought to examine how microglial cells respond to UCB toxicity in Gunn rats. We hypothesized that UCB toxicity induces microglia activation and that prolonged microglial activation plays a role that makes the Gunn rat suitable as an animal model of schizophrenia. We observed the morphological features, distribution, and ultrastructural characteristics of microglial cells in adult Gunn rats. We also determined the ratio of resting/ramified cells to activated cells and examined the neuron–microglia interactions. These studies were performed on the hippocampal dentate gyrus (DG), and the results were compared to those to Wistar rats as a normal control.

MATERIALS AND METHODS

The animals were male homozygous (j/j) Gunn rats and male Wistar rats (N = 10 each, Japan SLC, Inc.) that were 8 weeks old at the time of the experiments. Under deep intraperitoneal anesthesia, the rats were perfused transcardially. After perfusion, the brain was quickly removed, post-fixed in a solution of 4% paraformaldehyde at room temperature for 4h. Later, the brains were cut in at 50 μm thick in the frontal plane using a freezing mictrotome. Using immunohistochemical techniques, we compared the distribution, morphology, and ultrastructural features of microglial cells in Gunn rats with Wistar rats as a normal control. We used the antibody rabbit anti-Iba1 (1:4000) to determine the microglial cells, and mouse anti-CD11b (1:500) to determine the activated microglial cells. Later, using the Stereo Investigator system software, we measured the number of microglial cells, determined the ratio of activated and resting microglia and observed microglia-neuron interactions. We characterized the microglial cells in the hippocampal dentate gyrus. Later we performed the statistical analysis with the SPSS software. Differences between Gunn rats and controls were compared using the two-tailed Student's t-test with p of <0.005 considered to be a significant difference.

RESULTS AND DISCUSSION

First, we revealed that Iba1-labelled microglial cells showed activated morphology in the DG

of Gunn rats. Second, during ultrastructural observation, we found that these activated cells contained enlarged areas of cytoplasm rich in organelles, and that some of them formed phagocytic pouches or engulfed large phagocytic vacuoles. Third, there was significant difference in CD11b expression areas in the DG of Gunn rats compared to controls.

When the CNS is injured, microglia rapidly shifts into an activated state and migrate to the damaged sites. Activated microglia are marked by a number of characteristic events, affecting cellular morphology, cell size, cell number, and at the molecular level, the pattern of cell surface molecules expressed (immunophenotype) as well as the pattern of cytokines and growth factors produced, which distinguish them from the resting/ramified phenotype.

An additional highly characteristic feature of microglia activation is the remarkable capacity of the microglial cell population to expand, especially in response to acute injury. Our result showed no significant difference between Iba1-labeled microglial cell numbers in Gunn rats and in controls. However, we found a significant difference in the area of CD11b expression as marker of microglial activation. These results suggest that microglial cells in adult Gunn rats showed a feature of microglial activation without expansion of the cell population.

In homozygous (j/j) Gunn rats, few signs of bilirubin toxicity are present during the first postnatal weeks. Our previous study found that blood bilirubin levels in adult Gunn rats were still high and the presence of microglia activation suggested the possibility of chronic neuronal inflammation. When acute microglial activation becomes a chronic condition following injury, the microglial cells is potentially maladaptive or neuroprotective. We suggest that chronic microglial activation in adult Gunn rats is potentially more maladaptive than neuroprotective.

Prolonged microglia activation of microglia may lead to neuronal degeneration, white matter abnormalities, decreased neurogenesis, apoptosis, and brain damage, and may thus be one of the important factors in the pathophysiology of schizophrenia

CONCLUSION

We propose that activation of microglia could be an important causal factor of the behavioral abnormalities and neuropathological changes in Gunn rats. These findings may provide basic information for further assessment of the Gunn rat as an animal model of schizophrenia.