学 位 論 文 の 要 旨

氏名　古屋 智英

学 位 論 文 名　Yokukansan Promotes Hippocampal Neurogenesis Associated With the Suppression of Activated Microglia in Gunn Rat

発 表 雑 誌 名（巻，初頁～終頁，年）　Journal of Neuroinflammation 2013, 10:145

著 者 名　Motohide Furuya, Tsuyoshi Miyaoka, Toshiko Tsumori, Kristian Liaury, Sadayuki Hashioka, Rei Wake, Keiko Tsuchie, Michiyo Fukushima, Satoko Ezoe, Jun Horiguchi

論 文 内 容 の 要 旨

INTRODUCTION

Schizophrenia (SCZ) is one of the most intriguing psychological disorders; it has a huge adverse impact on QOL and requires high expenditure for treatment. Although the pathophysiology of SCZ is still far from being elucidated, researchers have indicated in hippocampal neurogenesis as a key target for the pathogenesis and treatment of this disease. There are ample evidences suggesting that SCZ is related to decreased hippocampal neurogenesis. Moreover, neuroinflammation associated with activated microglia is negatively correlated with hippocampal neurons. Furthermore, previous studies have suggested that adjuvant anti-inflammatory drugs are effective for treating SCZ.

From the standpoint of the heterogeneity of SCZ, previous studies have indicated a close association between unconjugated bilirubin (UCB) and SCZ. On the basis of these findings, we suggested that elevated levels of UCB play an important role in SCZ etiology, and that the Gunn rat, which exhibits a high concentration of UCB, is useful as an animal model of SCZ. Furthermore, our previous studies have shown that Gunn rats have cognitive deficits and contain activated microglia in the hippocampus.

We recently reported that yokukansan (YKS), which is a traditional Japanese medicine, is effective as an adjunctive therapy for SCZ. To date, basic studies have suggested that the both glutamate and serotonin signaling pathways are involved in the antipsychotic action of YKS. Furthermore, facilitated hippocampal neurogenesis may be associated with the anti-inflammatory action of YKS, as several ingredients of YKS possess potential anti-inflammatory properties.

In this study, we hypothesized that the YKS shows effectiveness in treating SCZ by promoting hippocampal neurogenesis and, in part, via its anti-inflammatory actions. We investigated whether YKS affects cognitive functions. To gain further insight into the neurobiological effects of YKS, microglial activation and neurogenesis in the hippocampus were evaluated using immunohistochemistry.

MATERIALS AND METHODS

The animals were 7 weeks old male homozygous Gunn Rats and male Wistar rats (N=8 each, Japan SLC, Inc.). Drug was used YKS (Tsumura & Co., Tokyo, Japan) is composed of 7 dried medical herbs. The dosage (1g/kg) of YKS used in this study was decided based on previous studies. The rats were divided into 4 groups. The rats in the control groups (Wistar - Control and Gunn-Control) were given drug-free water *ad libitum* for 6 weeks, whereas those in YKS-treated groups (Wistar - YKS and Gunn - YKS) were given water containing 0.6% YKS (corresponds to a dosage of 1g/kg of body weight) for the same period. Each group of animals received 50mg/kg bromodeoxyuridine (BrdU) 4 times a day during the 2 weeks of drug dosing. The OLT (object-location test), had been used to used test cognitive function, was performed on 6th week. The test was performed according to a procedure described previously. After the test was finished, the rats were perfused transcardially under deep intraperitoneal anesthesia. After perfusion, the brain was removed quickly and post-fixed. Later, the brains were cut in at 40 μm thick in the frontal plane using a freezing microtome. We used the antibody rabbit anti-Iba1 (1:4000, a marker of microglia/macrophages), mouse anti-CD11b (1:500, CD11b expression is up-regulated in activated microglia), rat anti-BrdU IgG (1:10) and mouse anti-NeuN (1:200). All sections were visualized using a confocal laser-scanning microscope and the Fluo-View software. The number of Iba1-labeled cells in the region of interest was calculated automatically by the Stereo Investigator soft ware Ver. 7.0. Sections containing CD-11b-labeled cells were analyzed using the Image J 1.46r software. Results were analyzed by one-way ANOVA and *post hoc* Bonferroni test to determine differences among groups. Values are expressed as the mean ± SEM. Analyses were performed using the SPSS software. In the analyses, P values < 0.05 were considered statistically significance.

RESULUTS AND DISCUSSION

The results are suggesting that cognitive function was disturbed in Gunn rats. But we found that YKS ameliorated spatial working memory in the Gunn rats. Furthermore, CD11b expression in Iba1-labeled microglial cells in the DG was compared between the GY and GC groups. Our results showed that, microglial cells in the GY group exhibited lower levels of CD11b immunoreactivity. To determine the survival of newly born neurons in the DG, the

number of NeuN+ cells among BrdU-labeled cells, YKS (GY group) significantly ameliorated the decrease in the number of cells observed in the GC group. YKS inhibited microglial activation and promoted neurogenesis in the hippocampal dentate gyrus of these rats. These results suggest that the ameliorative effects of YKS on cognitive deficits may be mediated in part by the suppression of the inflammatory activation of microglia.

The purpose of the present study was to determine whether chronic YKS treatment ameliorates the cognitive deficits associated with SCZ in Gunn rats. Furthermore, we investigated whether YKS suppresses microglial activation and promotes neurogenesis in the DG of Gunn rats. This study yielded 3 major findings, which are discussed below.

First, YKS ameliorated the cognitive dysfunction of Gunn rats. This finding suggests that YKS enhances the integration of newly cells into the existing neural network in the DG. The performance in the OLT relies on an intact DG, not other subregions. Accordingly, the OLT was used in the present study to determine if YKS restore the function of hippocampus through neurogenesis.

Second, YKS suppressed microglial activation in the DG of Gunn rats. We presume that this result reflects the potent anti-inflammatory activity of YKS. Our results showed that no significant difference between the numbers of microglia cells in Gunn rats compared to control. However, we found significant difference in microglial activation, which was suppressed by the administration of YKS. A recent meta-analysis study has suggested that anti-inflammatory drugs, such as COX-2 inhibitors, aspirin, and minocycline are effective in SCZ treatment. However, long-term use of these drugs increases the risk of complications, i.e., COX-2 inhibitors, aspirin, and minocycline induce adverse cardiovascular events, gastric ulcers and gastrointestinal bleeding, and emergence of drug-resistant strains of bacteria, respectively. Therefore, it is difficult to use these drugs in clinical treatment for a prolonged period. YKS has exhibited high safety and tolerability in several clinical studies.

Third, YKS promoted the survival of new neurons in the DG of Gunn rats. We suppose that this finding was, in part, due to the suppression of activated microglia in the DG by YKS. Several studies have suggested that the inflammation associated with activated microglia is detrimental to the survival of new hippocampal neurons. Therefore, we evaluated whether YKS affects hippocampal neurogenesis in the DG of Gunn rats.

CONCLUSION

To the best of our knowledge, this is the first *in vivo* study showing that YKS ameliorates cognitive dysfunction, suppresses microglial activation in the DG, and promotes hippocampal neurogenesis. The effects of YKS in SCZ may occur partly via the suppression of the inflammatory activation of microglia. Our results suggest a possible mechanism to explain the efficacy of YKS in SCZ and contribute to a better understanding of the pathophysiology of SCZ.