学 位 論 文 の 要 旨

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学 位 論 文 名	Effect of Ninjin'yoeito on Lipopolysaccharide-Induced Depressive-Like Behavior and Glial Activation in the Hippocampus
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論 文 内 容 の 要 旨

INTRODUCTION

Depression is the most common psychiatric disorder. Characteristic symptoms of depression include sad mood, despair, anhedonia, and, in cases of severe depression, suicide. Post-mortem studies suggest that glial pathological changes are involved in the hippocampus of depressed patients. Furthermore, glial cells were activated in animal models of depression. Lipopolysaccharide (LPS), a commonly used active inflammatory substance, is used in animal studies to induce depressive-like behavior related to glial cell activation.

Ninjin'yoeito (NYT), a Japanese herbal medicine, is a multi-component drug. Clinical studies reported that NYT improved depressive symptoms in Alzheimer's disease patients. In an animal model of depression, NYT improved depressive-like behavior induced by chronic corticosterone administration. Additionally, several studies reported that the active ingredients of NYT including ginseng, polygala root, or angelica root suppressed glial cell activation. These results suggested the potential of NYT to suppress glial cell activation-related depressive-like behavior.

In the present study, we investigated the effect of NYT treatment on depressive-like behavior and activated glial cells induced by LPS in rats. First, we conducted a forced swim test

(FST) as a model of despair, which is a commonly used test for animal depressive-like behavioral studies. Next, we examined the activation of microglia and astrocytes in the hippocampus of a rat model of depression.

MATERIALS AND METHODS

Eight-week-old Wistar male rats were used in the current study. Rats were divided into four groups (n = 6): sham, NYT, LPS, and NYT-LPS. NYT (2000 mg/kg body weight) or distilled water as a control were administered orally by intragastric gavage once daily for 16 days. LPS (*Escherichia coli* serotype 0111:B4; 1 mg/kg body weight) or saline as a control were administered after NYT via intraperitoneal injection every other day for a total of eight times.

A behavioral test (FST) was performed on days 16 and 17. Next, brain samples were collected immediately after completing the behavioral tests for immunohistochemistry analysis. Microglial cell activation was evaluated by staining for ionized calcium-binding adaptor molecule 1 (Iba1) and glial fibrillary acidic protein (GFAP) staining was used to evaluate activated astrocytes. Free-floating brain tissue sections were incubated with the following primary antibodies: rabbit anti-Iba1 (1:2000) and goat anti-GFAP (1:2000) overnight. Next, the sections were incubated with biotinylated anti-rabbit (or anti-goat) IgG antibody (1:1000) for 1 h at room temperature.

Immunohistochemistry images were captured with a 20× objective lens at three areas of interest in the hippocampal formation, dentate gyrus (DG), cornu ammonis (CA) 1, and CA3 regions. Specific areas were traced and captured manually using ImageJ software for analysis by a trained observer in a blinded manner. Twenty images were obtained bilaterally from each area, and 60 images were analyzed from each rat. All immune-labeled elements were automatically converted beyond the threshold range to pure black pixels, and then the remaining image area was converted to pure white pixels. The percentage of pure black pixels was calculated automatically by ImageJ software for statistical analysis.

All experiments with animals in this study were approved by the Animal Care and Use Committee of Shimane University (Authorization No: IZ2-104). All data are presented as the mean ± standard error of the mean (S.E.M.). We used a one-way analysis of variance followed by post hoc Tukey's honestly significant difference test to evaluate differences between groups. Statistical analyses were performed with SPSS software and the p-values < 0.05 were considered statistically significant.

RESULTS AND DISCUSSION

The immobility time of rats was increased significantly in the LPS group compared with the sham and NYT groups. Furthermore, the NYT-LPS group showed a significant decrease in

immobility time compared with the LPS group. These findings suggest that NYT improved depressive-like behavior (despair behavior). Meanwhile, NYT did not significantly recover the bodyweight gain inhibited by LPS. In addition, NYT treatment significantly reduced Iba1 expression in the hippocampal DG. Quantification analysis showed that Iba1 immunoreactivity was increased significantly in the DG, CA1, and CA3 regions in the LPS group compared with the sham and NYT groups. Furthermore, the NYT-LPS group had significantly decreased Iba1 immunoreactivity in the DG region compared with the LPS group. However, NYT did not affect GFAP expression in the hippocampus, suggesting NYT did not inhibit astrocyte activation induced by LPS.

NYT was reported to improve the immobility time of C57BL/6 mice chronically administered corticosterone in the FST. Among the components of NYT, gomisin N, the active substance of schisandra fruit, was reported to ameliorate the performance of male ddY mice in the FST by attenuating inflammation. Previous studies have reported that glial cell activation causes neuroinflammation in animal models of depression. Indeed, another active component of NYT, tenuigenin (the active substance of the polygala root), inhibited microglial activation and suppressed the release of proinflammatory cytokines in an animal model of Parkinson disease induced by LPS. Thus, our results suggest that NYT may decrease the release of proinflammatory cytokines to attenuate microglial activation in the hippocampus, which may allow recovery from depressive-like behavior. However, we found that NYT did not affect astrocyte activation in the hippocampus. This result is in accordance with a previous study reporting that NYT treatment had no effect on astrocyte cell numbers in the hippocampus DG of male C57BL/6 mice induced by corticosterone.

On the basis of these findings, the therapeutic effect of NYT or its components may improve hippocampal neuroinflammation in animal models of depression induced by LPS, and subsequently allow recovery from depressive-like behavior.

CONCLUSION

In conclusion, the present study shows that NYT alleviates depressive-like behavior in rats. This effect may be related to the attenuation of glial cell activation in the hippocampus of a rat model of depression induced by LPS. These findings may provide crucial information to clarify the role of pathological changes in glia during the pathogenesis of depression.