学 位 論 文 の 要 旨

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学 位 論 文 名　Systemic Administration of Porphyromonas Gingivalis Lipopolysaccharide Induces Glial Activation and Depressive-Like Behavior in Rats

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論 文 内 容 の 要 旨

INTRODUCTION

Major depression (MD) is a prevalent life-threatening psychiatric disorder. Approximately 322 million people are affected by MD worldwide. The neuroinflammatory process in which activated microglia and astrocytes generate excessive pro-inflammatory cytokines has been implicated in the pathogenesis of MD. Increasing evidence also suggests that infection and persistent low-grade inflammation in peripheral tissues play a role in MD. Increasing evidence also suggests that infection and persistent low-grade inflammation in peripheral tissues play a role in MD. Periodontitis is one of the most common chronic inflammatory diseases in adults. Although recent systematic reviews and meta-analyses have suggested a mutual relationship between periodontitis and MD, the biological mechanisms by which periodontitis instigates MD are unclear.

Several animal studies have demonstrated that peripheral administration of lipopolysaccharide (LPS) from *Escherichia coli* increases the levels of pro-inflammatory cytokines in both the periphery and brain and induces abnormal behavior similar to MD, called depressive-like behavior. It has also been believed that systemic inflammation causes the neuroinflammatory process through several pathways. It is proposed that such neuroinflammation, in turn, elicits MD symptoms by influencing brain functions, especially neurotransmitter metabolism. We investigated whether a systemic administration of lipopolysaccharide (LPS) from *Porphyromonas gingivalis (Pg)*, a major Gram-negative pathogen of periodontitis, causes depressive-like behavior and glial activation in the hippocampus and the prefrontal cortex (PFC), which are MD-related brain regions.

MATERIALS AND METHODS

Eight-week-old male Sprague Dawley rats were randomly divided into a behavioral test group and an immunohistochemistry group. The rats in each group were further assigned to the sham injection (saline) and *Porphyromonas gingivalis*-lipopolysaccharide (*Pg*-LPS) injection protocols. The number of individuals in each group was 5 or 6. The rats received an intraperitoneal injection of saline or *Pg*-LPS with gradually increasing doses (day 1: 0.5, day 2: 0.5, day 3: 0.75, day 4: 0.75, day 5: 1.0, day 6: 1.0, and day 7: 1.0 mg/kg of body weight) for seven consecutive days.

After the systemic administration, the behavior test group underwent the forced swimming test (FST) and Y-maze test. The Y-maze test was performed 2 hr after the last i.p. injection. 2 hr after the Y-maze test, habituation of the forced swimming test (FST) was carried out.

For the immunohistochemistry group, we quantified the immunoreactivity for microglial Iba-1 (ionized calcium-binding adapter molecule 1) and astrocytic glial fibrillary acidic protein (GFAP) in the hippocampus (dentate gyrus [DG], cornu ammonis [CA1 and CA3]) and PFC (prelimbic [PrL] and the infralimbic [IL]) areas.

All experiments with animals in this study were approved by the Animal Care and Use Committee of Shimane University (Authorization No: IZ3-50). All the data are expressed as the mean ± standard deviation (S.D.). For two group comparisons, we performed the independent sample t-test. The statistical analysis was conducted by using the software SPSS (version. 23.0, IBM Corp., Tokyo, Japan). A *p*-value less than 0.05 was considered significant.

RESULTS AND DISCUSSION

In the FST, learned helplessness in the rats was evaluated by measuring immobility time, which was 108.73 ± 29.33 sec in the sham group (n = 6) and 181.54 ± 49.20 sec in the *Pg*-LPS group (n = 5). The immobility time in the *Pg*-LPS group was significantly longer than that in the sham group.

We evaluated the rat's spatial working memory in the Y-maze test by calculating the percentage of SAB. The percentage of SAB was 72.83 ± 12.29% in the sham group (n = 6) and 57.43 ± 4.75% in the *Pg*-LPS group (n = 5). A significant reduction in the % SAB was detected in the *Pg*-LPS group compared to the sham group.

We investigated the immunoreactivity of microglial Iba-1 in the hippocampus (CA1, CA3, and DG regions) and the PFC (PrL and IL regions). Among these regions, the CA3 region showed significantly higher immunoreactivity for Iba-1 in the *Pg*-LPS-injection group (18.05 ± 2.49%, n = 5) compared to the sham injection group (13.95 ± 1.57%, n = 5). Also, the PrL region showed significantly higher immunoreactivity for Iba-1 in the *Pg*-LPS-injection group (18.05 ± 1.73%, n = 5) compared to the sham injection group (13.49 ± 2.31%, n = 5). In the other regions, the *Pg*-LPS group (n = 5) showed a non-significant trend for higher Iba-1 immunoreactivity compared

with the sham group (n = 5).

We also evaluated the astrocytic GFAP immunoreactivity in the hippocampus (CA1, CA3 and DG regions) and the PFC (PrL and IL regions). The hippocampal CA1 regions showed significantly higher immunoreactivity for GFAP in the *Pg*-LPS-injection group (27.98 ± 2.69%, n = 5) compared to the sham injection group (21.75 ± 4.88%, n = 5) and CA3 regions showed significantly higher immunoreactivity for GFAP in the *Pg*-LPS-injection group (21.47 ± 1.13%, n = 5) compared to the sham injection group (14.41 ± 3.21%, n = 5). Also, the DG region showed significantly higher immunoreactivity for GFAP in the *Pg*-LPS-injection group (31.19 ± 2.10%, n = 5) compared to the sham injection group (22.59 ± 4.67%, n = 5). On the other hand, in the PFC PrL and IL regions, we observed no significant difference in GFAP immunoreactivity between the *Pg*-LPS (n = 5) and sham groups (n = 5).

The immobility time represents learned helplessness and can be considered behavioral despair. This finding is consistent with an earlier investigation which demonstrated that mice treated intraperitoneally with *Pg*-LPS exhibited significantly prolonged immobility time in the FST. The decrease in SAB can be interpreted as impaired spatial working memory. Clinically, it has been observed that individuals with MD tend to have considerably impaired working memory. Our present finding is also in line with a report that peripheral injection of *Pg*-LPS led to impaired spatial learning in mice.

Our finding of astrocytic activation in the hippocampal regions, as shown by raised GFAP immunoreactivity, is consistent with the above-cited studies using mice, which demonstrated that i.p. injection of *Pg*-LPS raised the number of GFAP-immunopositive cells in the hippocampus and PFC. Our finding of *Pg*-LPS-induced activation of microglia in the CA3 and PrL regions was verified by quantification of Iba-1 immunoreactivity, while an aforementioned study described microglial activation based only on morphological inspection.

It is elusive whether glial activation is exactly involved in the MD pathogenesis, as only a few studies have evaluated glial activation using postmortem brain samples from individuals with MD. Nevertheless, *in vivo*, studies demonstrated that mice with activated microglia and activated astrocytes, which were induced by peripheral injection of *E. coli*-LPS, exhibited depressive-like behavior. Our present finding verified that *Pg*-LPS also caused microglial activation, astrocytic activation, and depressive-like behavior in rats.

CONCLUSION

Our major finding is that a repeated systemic administration of *Pg*-LPS elicited depressive-like behavior, and both microglial and astrocytic activation could be biological evidence for a causal relationship between periodontitis associated pathogens and MD.