主論文の要旨

Dimethyl fumarate improves cognitive impairment and neuroinflammation in mice with Alzheimer's disease

（フマル酸ジメチルはアルツハイマー病モデルマウスにおける認知機能の低下と神経炎症を改善する）

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【Introduction】

Alzheimer's disease (AD) is the most common cause of dementia affecting elderly individuals. Accumulation of amyloid-β (Aβ) and phosphorylated tau is a well-known neuropathological hallmark of AD. On the other hand, neuroinflammation, defined as an inflammatory response of the central nervous system (CNS), is mediated by activation of the innate immune system of the brain. Nuclear factor-erythroid 2-related factor 2 (Nrf2), a transcription factor encoded by *NFE2L2*, is a member of a small family of basic leucine zipper proteins. Nrf2 target genes are involved in inflammation, detoxification reactions, redox balance and energy metabolism, and proteostasis, all of which have been implicated in the pathomechanism of neurodegenerative diseases. Dimethyl fumarate (DMF), an approved drug for multiple sclerosis, can allow Nrf2 translocation to the nucleus and Nrf2 transactivation of target genes by binding to their AREs. Whether DMF-mediated activation of the Nrf2 pathway confers neuroprotection in mice with AD by controlling neuroinflammation and its detailed molecular basis remains to be determined.

【Methods】

We pretreated primary astrocytes with DMF and then administered IL-1α, TNFα, and C1q to induce inflammatory (A1) astrocytes. We measured the expression of Nrf2 downstream genes and A1 astrocyte markers using quantitative PCR (qPCR).

We orally administered DMF (300 mg/kg) to 6-month-old $App^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}F}$ (App-KI) and wild type (WT) control mice for 1 or 5 months. Four groups of mice (vehicle-WT, DMF-WT, vehicle-App-KI, and DMF-App-KI) were evaluated by behavioral experiments at 11 months of age. We sequentially isolated $Cd11b^{+}$ microglia and $ACSA2^{+}$ astrocytes from the cerebral cortices of 11-month-old mice using magnetic-activated cell sorting (MACS) and then examined the gene expression in glial cells using quantitative RT-PCR. In addition, we evaluated the effect of DMF on dystrophic neurites and Aβ accumulation using immunohistochemistry or immunoblotting.

All data are expressed as means ± SEM. One-way or two-way ANOVA with or without repeated measures was used, followed by Tukey's test when F ratios were significant ($p < 0.05$). Significant differences between two groups were assessed using the Student's t-test.

【Results】

DMF significantly increased mRNA expression of Nrf2 downstream genes such as *Hmox1* and *Gclm* and downregulated the mRNA expression of inflammation-related molecules such as *H2-d*, *H2t23*, *C3*, and *Socs3* by activating the Nrf2 pathway in proinflammatory (A1) astrocytes (Fig. 1A and B). In Nrf2-deficient astrocytes, the expression of the A1 astrocyte markers *H2-d* and *H2t23* and that of *C3* and *Socs3* were unaffected by DMF treatment (Fig. 1A and C).

To identify the glial cell type that responds to DMF treatment *in vivo*, we orally administered DMF to 6-month-old $App^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}F}$ (App-KI) and WT mice for 1 month. We isolated microglia and astrocytes from the cerebral cortices by MACS and measured the expression of Nrf2 (*Nfe2l2*) mRNA and its downstream genes using qRT-PCR (Fig. 2A). Intriguingly, we found that the expression of Nrf2-downstream genes was different between microglia and astrocytes after DMF administration. In isolated microglia, only *Gclm* expression was upregulated after DMF treatment (Fig. 2B), whereas *Nqo1* and *Osgin1* expression was upregulated in the astrocytes of DMF-administered App-KI mice (Fig. 2C).

To determine the effects of DMF on the cognitive function and neuroinflammation in App-KI mice, we orally administered DMF to 6-month-old App-KI and WT mice for 5 months (Fig. 3A). We did not find that DMF administration affected spontaneous behavior in mice using an open field test (Fig. 3B and 3C). In the novel object recognition test, vehicle-administered App-KI mice showed significantly less exploratory preference for the novel object than WT mice in the test session, as expected. DMF administration significantly improved the preference index in App-KI mice (Fig. 3D and 3E).

To further investigate the molecular basis of the beneficial role of DMF in *App*-KI mice, we sequentially isolated $Cd11b^{+}$ microglia and $ACSA2^{+}$ astrocytes from the cerebral cortices of 11-month-old mice using MACS and then examined the gene expression in glial cells using qRT-PCR (Fig. 4A). We found that DMF administration upregulated *Osgin1* and downregulated the expression of the disease-associated microglia (DAM) marker *Cd11c*, complement *C1qa*, and its receptor *C3ar1* in App-KI microglia (Fig. 4B). We also examined the expression levels of reactive astrocyte markers and neuroinflammation related genes, found that the levels of *H2d*, *C3* and *Stat3*, which were upregulated in App-KI astrocytes, were suppressed by DMF administration (Fig. 4C).

To confirm that DMF reduces neuroinflammation in App-KI mice, we performed immunofluorescence analysis of the cerebral cortices of DMF- or vehicle-administered WT and App-KI mice. Chronic administration of DMF significantly inhibited the expression of GFAP and IBA1 in App-KI mice (Fig. 5A, B, and D). Furthermore, the immunoreactivity of C3 and pSTAT3 mainly overlapped with GFAP-positive astrocytes in App-KI mice and was substantially reduced with DMF administration (Fig. 5A, B, and D). In contrast, no significant difference in Aβ immunoreactivity was observed between App-KI mice with and without DMF administration (Fig. 5C, D). DMF administration reduced BACE1-positive dystrophic nerve terminals in App-KI mice (Fig. 5C, D). The protein level of p-STAT3 was significantly decreased in DMF-administered App-KI mice, indicating that oral administration of DMF inhibited the STAT3 pathway (Fig. 5C, D).

【Discussion】

Chronic oral DMF administration conferred neuroprotection in App-KI mice with improved

performance in the novel object recognition test and reduced dystrophic neurites. Our gene expression analysis of MACS-isolated microglia and astrocytes revealed that DMF attenuated the expression of proinflammatory genes, particularly in the astrocytes of App-KI mice. Furthermore, our gene expression analysis also revealed that the expression of Nrf2 target genes, *Hmox1, Gclm, Nqo1*, and *Osgin1*, was increased in cortical astrocytes isolated from DMF-administered App-KI mice. Although previous studies have demonstrated anti-inflammatory effects of DMF in astrocytes via the Nrf2 pathway, we found that the expression of *C3* and *C3ar* was significantly suppressed in astrocytes and microglia isolated from DMF-administrated App-KI mice, respectively. These results suggest that astrocyte–microglia interaction contributes to complement inhibition in AD mice. Moreover, DMF had a beneficial effect on C3 expression and, through C3-mediated STAT3 signaling, inhibited *Stat3* and its downstream gene *Socs3* in mice with AD. Reducing STAT3 signaling by inhibiting C3–C3aR signaling alleviates neuroinflammation in an App-KI model of AD.

【Conclusions】

Our study identified the activation of astrocytic Nrf2 signaling as a viable therapeutic target in AD by controlling neuroinflammation, particularly through the regulation of C3-STAT signaling in astrocytes. Furthermore, it raised the possibility of repositioning DMF as a drug for treating neurodegenerative diseases, including AD.