Increased arachidonic acid-containing phosphatidylcholine is associated with reactive microglia and astrocytes in the spinal cord after peripheral nerve injury.

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論文題目

Increased arachidonic acid-containing phosphatidylcholine is associated with reactive microglia and astrocytes in the spinal cord after peripheral nerve injury.

(末梢神経損傷後のアラキドン酸含有ホスファチジルコリンの増加は脊髄内の活性化ミクログリアとアストロサイトに関連している)

論文の内容の要旨

[Introduction]

Peripheral nerve injury (PNI) provokes changes in neuronal, glial and immune interactions within the spinal cord. Although the alteration of neuropeptides, proteins and transcription factors in the spinal cord following spinal nerve transection (SNT) have been extensively studied in recent years, changes in polyunsaturated fatty acid-containing phosphatidylcholines (PUFA-PCs), including arachidonic acid-containing phosphatidylcholines (AA-PCs) and docosahexaenoic acid-containing phosphatidylcholines (DHA-PCs), in the spinal cord after PNI remains unknown. Microglia and astrocytes, which are the main glial cells in the central nervous system (CNS), are rapidly activated after PNI. However, the association between PUFA-PCs and the glial cells remain unknown. In order to clarify these questions, we investigated the changes of AA-PCs, DHA-PCs in relation to the glial cells in the spinal cord following SNT using matrix-assisted laser desorption/ionization mass spectrometry (MALDI-IMS) analysis and immunohistochemistry.

[Materials and Methods]

All experiments were conducted according to protocols approved by the Animal Care and Use Committee of the Hamamatsu University School of Medicine.

Eight-week-old C57BL/6JJmsSlc female mice were used for this study. The left sciatic nerve of the mice in SNT group (n = 21) was transected under anesthesia. Sham-operated mice (n = 21) underwent nerve exposure without transection of the sciatic nerve. L3-L5 lumbar spinal cord segments were harvested at 3, 7 and 28 days post-operatively. Tissues were sliced into 10 μm thick axial serial sections for MALDI-IMS analysis and immunohistochemistry.

[Results]

We first compared the ion abundances between the sham and the SNT group using MALDI-IMS 7 days post-operatively. We found that $[PC(16:0/20:4)+K]^+$ was increased in both the ipsilateral laminae IX of the ventral horn and the laminae I-III of the dorsal horns 7 days after SNT.

We used anti-Iba1 and anti- Glial fibrillary acidic protein (GFAP) antibodies to analyze

distributions of microglia and astrocytes in the spinal cord after SNT. We found that the Iba1 positive and GFAP positive cells were significantly higher in the SNT mice than in the sham. Moreover, the locations of Iba1 positive microglia and GFAP positive astrocytes resembled the areas highly expressing $[PC(16:0/20:4)+K]^+$. To confirm the activation of microglia, we performed Major Histocompatibility Complex II (MHCII) and CD86 staining to identify the activated and non-activated microglia. We found that Iba1 positive microglia also became immuno-positive for MHCII and CD86 staining, confirming the activation of the microglia.

We additionally analyzed the spinal cord on days 3 and 28 days post SNT. The high immunoreactivities of Iba1 and GFAP were observed in the ventral and dorsal horns from day 3 to 28 together with elevated expression of $[PC(16:0/20:4)+K]^+$.

We also evaluated the expression of DHA-PCs ($[PC(16:0/22:6)+K]^+$, $[PC(18:1/22:6)+K]^+$ and $[PC(18:0/22:6)+K]^+$) at 28 days post SNT. However, none of the DHA-PCs showed any changes after SNT.

[Discussion]

In the present study, we focused on analyzing spatial distribution of the PUFA-PCs containing AA-PCs and DHA-PCs in the spinal cord after SNT using MALDI-IMS.

PUFA-PCs are associated with cell types, and influence the properties of membranes. Microglia and astrocytes, which are the main glial cells in the CNS, immediately react to PNI. The similar spatiotemporal expression pattern was observed between $[PC(16:0/20:4)+K]^+$ and activated microglia and astrocytes. Microglia are morphologically and functionally dynamic cells which have different morphological phenotypes. Cellular membranes are constructed by lipids, especially the rich-content phospholipids which are required for shape changes. Therefore, we speculated that $[PC(16:0/20:4)+K]^+$ may be associated with reactive microglia via increasing AA-PC composition in the microglia membrane.

We also found the reactive astrocytes occurred in the region of increased $[PC(16:0/20:4)+K]^+$ expression and microglia activation. We hypothesize that the activation of microglia resulted in the elevation of $[PC(16:0/20:4)+K]^+$ and the activation of astrocytes, which further led to the increase in $[PC(16:0/20:4)+K]^+$. In our spatiotemporal study, we observed increased microglia, astrocytes and the expression of $[PC(16:0/20:4)+K]^+$ persisting until 28 days after SNT, which could be reflecting the development and maintenance of neuropathic pain, or neurodegeneration.

As indispensable membrane phospholipids, DHA-PCs are required in motor neurons. $[PC(16:0/22:6)+K]^+$ was found to be enriched in the large motor neurons of the ventral horn of the spinal cord. However in our study, neither DHA-PCs nor NeuN positive cell showed significant change in the spinal cord 28 days after SNT. These results suggested

that the preservation of neuron in the mouse spinal cord.

[Conclusion]

We conclude that $[PC(16:0/20:4)+K]^+$ is associated with the reactive microglia and astrocytes in the spinal cord after PNI and further investigation is required to identify the exact cell sources.