(様式4) (Form4)

学 位 論 文 の 内 容 の 要 旨

Dissertation Abstract

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(学位論文のタイトル) Title

Suppression of Neuropeptide by Botulinum Toxin Improve Imiquimod-induced Psoriasis-like Dermatitis via the Regulation of Neuroimmune System

(神経免疫機能を介したボツリヌス毒素による乾癬の病態制御機構の解明)

(「論文目録（様式3）」の主論文の部分を記載する。英文の場合は和訳をつける。)

For English paper, Japanese title is necessary.

(学位論文の要旨) 2,000字程度、A4判 (approx.800 Words in English /A4 size)

Background: Psoriasis is a multifactorial skin disease arises from a complex interaction of genetics, immune system, and environmental aspects. In psoriasis, the process of keratinocytes maturation is accelerated and cause the immature cells to accumulate and form an erythematous silvery, thick scales with well-demarcated border. Among several type of psoriasis, psoriasis vulgaris is the most common type and mostly found on the scalp, extensor area, or body trunk. IL-23/Th17 immune axis has been considered as a primary modulator in psoriasis. Over the last decades, the role of immune system in the development of psoriasis has significantly expanded. Several findings imply that nervous system may play a role in psoriasis. Studies showed that the number of $PGP9.5^{+}$ nerve fibers and their secreted neuropeptide, such as substance P (SP) and calcitonin gene-related product (CGRP) were increase in psoriatic lesion. Moreover, psoriasis is often associated with emotional stress and study showed that psychological stress could increase the secretion of SP and CGRP. SP and CGRP might induce neurogenic inflammation, such as psoriasis. A substance that inhibits the release of neurotransmitter, such as Botulinum toxin (BTX) might improve the lesion. Several cases reported an improvement on psoriatic lesion following BTX injection. However, the role of neuropeptide and the effect of BTX on psoriasis has not been fully clarified.

Objective: We aim to ascertain the role of neuropeptide secreted for neurons in the pathogenesis pathway and the suppression effect of BTX-B in psoriasis *in vivo*

Method: Psoriasis-like dermatitis was induced by applying an Imiquimod (IMQ) cream on the back skin of the mouse for 6 consecutive days. To investigate the effect of BTX-B on the development of psoriasis-like dermatitis, a diluted BTX-B was injected intradermally 24hr before IMQ treatment. Skin dermatitis was evaluated using Psoriasis Severity Index (PSI) score. The infiltrating inflammatory cells and cytokines production at the lesional skin area were assessed by immunostaining and real-time PCR. We also tested the effect of selective CGRP antagonist ($CGRP_{8-37}$) on psoriasis-like

dermatitis in IMQ-treated mice.

Result: BTX-B injection ameliorate skin dermatitis in IMQ-induced psoriasis-like mice model. Erythema, skin scales, and skin thickness of PSI score was significantly lower in BTX-treated mice. Histological examination showed the changes that often seen in psoriatic skin, including hyperkeratosis, acanthosis, and elongation of epidermal rete ridges were inhibited by BTX-B injection. The measurement of epidermal thickness was significantly lower in treated mice compared to control mice. The number of infiltrated $CD3^+$ and $CD4^+$ T cells, $CD11c^+$ dendritic cells, and mast cells were significantly reduced following BTX-B injection. Cytokine production of IL-17A/F in lesional skin was significantly inhibited by BTX-B injection. Additionally, the expression of $PGP9.5^+$ nerve fibers and neuropeptides (SP and CGRP) were significantly inhibited. Next, we also observed the effect of $CGRP_{8-37}$ on psoriasis-like dermatitis in IMQ-treated mice. In this study, a similar effect was observed after the injection of $CGRP_{8-37}$. $CGRP_{8-37}$ suppressed the development of skin dermatitis and lowering the PSI score compared to the control mouse. The epidermal thickness significantly reduced following $CGRP_{8-37}$ injection. Furthermore, $CGRP_{8-37}$ also significantly inhibited the infiltration of inflammatory cells ($CD3^+$, $CD4^+$, $CD11c^+$) and the secretion of IL-17A/F.

Discussion: Immune system is considered as a primary modulator in psoriasis. Inflammatory cytokines, such as TNF-α and IL-23 induced the inflammatory process in keratinocytes which leads to the formation of epidermal hyperplasia, acanthosis, and hyperkeratosis. Moreover, the increased number of nerve fibers and the production of neuropeptide (SP and CGRP) in psoriatic lesion imply the important role of nervous system. BTX is a neurotoxin that has been used as a treatment for variety of diseases. BTX-B cleave the VAMP of SNARE protein complex and several studies showed that BTX-B gives an earlier effect compared to BTX-A. The disruption of protein complex formation by BTX lead to the inhibition of neurotransmitter production. SP and CGRP might act synergistically as a mediator for the inflammatory process. It has been reported that CGRP released from nerve terminal is important to induce type 17 inflammation. CGRP activates dendritic cell and increase the production of IL-17 by promoting the transformation of naïve T cell into Th17 cell. In addition, SP and its receptor NK1R were upregulated in psoriatic patients. SP mediates the itch sensation and its involvement in scratching behavior has been reporter in mice model. It induces the activation and degranulation of mast cells. By injecting BTX-B, we could suppress the production of CGRP and SP which inhibits nerve elongation, the infiltration of immune cells (T cell and DCs) and the production of IL-17A/F. These might lead to the improvement of psoriatic lesion. Selective CGRP antagonist ($CGRP_{8-37}$) also significantly inhibit the development of psoriasis-like skin dermatitis in our mice model. Due to the effect of BTX-B in suppressing the production both SP and CGRP, it might give a greater suppressive effect in psoriasis-like skin dermatitis compared to CGRP antagonist alone.