(様式4) (Form4)

学 位 論 文 の 内 容 の 要 旨

Dissertation Abstract

（ HRITU BARAL ）Name 印

(学位論文のタイトル) Title

Inhibition of skin fibrosis in systemic sclerosis by botulinum toxin B via the suppression of oxidative stress

(B型ボツリヌス毒素は酸化ストレスを抑制することで
全身性強皮症モデルマウスにおける皮膚線維化を抑制する)

(「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: Systemic sclerosis (SSc) is an immune-mediated connective tissue disorder of unknown etiology which typically results in fibroblast activation with fibrosis of the skin and visceral organs and vasculopathy. Several studies over the years have reported that oxidative stress play a crucial role in the pathogenesis of skin fibrosis in systemic sclerosis (SSc). The therapeutic potential of various anti-oxidative agents in SSc have also been studied in the past. Regarding botulinum toxin (BTX) and oxidative stress, we previously identified that BTX injection suppresses pressure ulcer formation in cutaneous ischemia-reperfusion injury mouse model by regulation of oxidative stress. Additionally, we identified that BTX-B injection suppressed the activity of Raynaud's phenomenon and digital ulcers in SSc patients by clinical trial. However, to the best of our knowledge, the role of BTX-B in the regulation of bleomycin-induced oxidative stress in SSc mice model is still unclear and the therapeutic possibility of BTX administration for preventing skin fibrosis in SSc has not been studied before.

Objective: The main aim of our study was to investigate the effect of BTX-B on skin fibrosis in murine model of SSc and further investigate the underlying mechanism on the suppressive action of BTX-B both *in vivo* and *in vitro*.

Methods: Dermal fibrosis was induced in 8-week-old C57BL/6 or OKD48 mice with injections of bleomycin. Injections

of 300 µl of bleomycin at a concentration of 1 mg/ml were given 5 times per week for 2 weeks. To examine the effect of BTX-B, mice were injected with single dose of BTX-B (1U/mouse) subcutaneously, 24 hours before initiating the bleomycin induced fibrosis. For the observation of the luminescent signal, OKD48 (Keap1-dependent oxidative stress detector, NO-48) transgenic mice that have transgene encoding a modified Nrf2, which is an essential transcription factor for the expression of anti-oxidative stress genes were used. Since visible skin thickening was observed on 4-5days of bleomycin injection, OKD48 mice were examined after only 5 days of bleomycin (330 µl) injection. Similarly, for the examination of apoptotic cells in the injected skin and for the quantification of protein expression (HO-1, Caspase-3) in the lesional skin of mice, 5days injection of bleomycin was followed. Finally, the skin sampled from the injected site was collected for further investigations including H/E, masson-trichome and immuno-histochemical staining, rt-PCR, western blot analysis, apoptosis assay and so on. For *in vitro* studies, human dermal fibroblast obtained from dorsal forearm of diffuse cutaneous type SSc patients was used.

Results: We found that BTX-B injection significantly reduced dermal thickness revealed by H&E staining. Collagen content shown by masson-trichome staining and soluble collagen amount calculated by sircol collagen assay showed significant reduction in the BTX-B group. Similarly, the infiltration of inflammatory cells observed in bleomycin-induced skin fibrosis lesion in mice was also significantly reduced by BTX-B. We also identified that oxidative stress signal detected through bioluminescence in OKD48 mice after bleomycin injection in the skin was significantly decreased by BTX-B treatment. Additionally, mRNA levels of oxidative stress associated factors (NOX2, HO-1, Trx2) were significantly decreased by BTX-B. Likewise, apoptotic cells in the lesional skin of bleomycin-treated mice were significantly reduced by BTX-B. In addition, oxidant-induced intracellular accumulation of reactive oxygen species in SSc fibroblasts was also suppressed significantly by BTX-B treatment.

Conclusion: Taken together, our study demonstrated the inhibitory effect of BTX-B on the production of collagen by possible reduction of inflammation, oxidative stress and oxidative cellular damage in bleomycin-induced murine model of skin fibrosis. Therefore, this study suggests that BTX-B could be possible therapeutic option for skin sclerosis in SSc patients. However, the mechanism of suppression of oxidative stress by BTX-B still remains to be elucidated, and further investigation is warranted in the future.