The Effects of Botulinum Toxin A on Fibrotic Activity and Cell Cycle Modulation in Keloid Fibroblast

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INTRODUCTION: There are many studies showing antifibrotic effect of various methods in keloid fibroblast.[1] The purpose of this study was to find out the antifibrotic effect and cell cycle modulatory effect of botulinum toxin A(BoTA) in keloid fibroblast. In order to reveal the effects, we have evaluated; 1)changes of collagen synthesis and degradation by BoTA[2], 2)effects of BoTA under fibrotic stimulation condition by TGF-β, and 3)effects on the cell cycle and apoptosis of keloid fibroblast[3]

MATERIALS AND METHODS: Human keloid fibroblasts were treated by 0, 1, 5, 10 uint/10^5cells of BoTA (Allergan, Irvine, CA) for 48hrs with and without 10ng/ml TGF-β. Type I and III collagen were analyzed quantitatively by RT-PCR. Time dependent effect of BoTA on MMP-1,2,9, TIMP-1 were examined by RT-PCR and gelatin zymogram. Changes of cell cycle were evaluated by flow cytometry(FACS analysis). The effect on apoptotic activity was evaluated by quantitative analysis of p53 and Bcl-2 through western blot. The effect on cell cycle modulation factors was analyzed by western blot of p21 and cyclin B.

RESULTS: When TGF-β was added simutaneously with BoTA, type III collagen mRNA expression significantly decrease in the keloid fibroblasts ($p<0.05$), however, type I collagen expression was not affected by BoTA. MMP-1 mRNA expression increased by BoTA, however, was not affected by adding TGF-β. MMP-2 activities increased by BoTA and even after adding TGF-β. TIMP-1 and MMP-9 activities were not affected by BoTA. The flow cytometric analysis for BoTA-induced cell cycle in keloid fibroblast showed the resting phase cells under BoTA were more than those in control.(Control: G0-G1:73.49%, G2-M:13.47%, S:13.04%. BoTA: G0-G1:75.67%, G2-M:16.56%, S:7.76%) p53, an apoptotic protein, showed significant increase in a dose-dependent fashion by BoTA under TGF-β(10ng/mL) ($p< 0.05$). p21, a cell cycle regulatory protein, showed dose-dependent increase by BoTA. However, Bcl-2 and cyclin B1 significantly decreased as antagonistic effects against p53 and p21.

CONCLUSION: BoTA has an inhibitory effect on type III collagen synthesis and can increase degradation of extracellular matrix. G0-M phase of keloid fibroblast was prolonged due to changes of cell cycle affected by BoTA. Additionally, BoTA can increase apoptosis of keloid fibroblasts but this effect can be promoted in just active proliferation phase of keloid.

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