

How Will Thrombin Affect the Expression of mRNA for Growth Factors in Corneal Cells?

Abstract

When the cornea is wounded, cells in the stroma divide and convert into two phenotypes, fibroblasts and myofibroblasts, which move into the injured area and participate in the wound healing process. Early in wound healing, thrombin is generated in the cornea and fibrin is laid down. Thrombin signals cells by binding to the Protease Activated Receptors (PAR), which may influence the cellular production of growth factors and cytokines. Growth factors of interest in our system include connective tissue growth factor (CTGF), cysteine-rich protein 61 (CYR61), transforming growth factor beta (TGF- β), and Interleukin 6 (IL-6). To test thrombin's effect on the expression of mRNA for these growth factors, 0, 0.1, or 1 unit/ml of thrombin was added to cultures of human corneal fibroblast and myofibroblast cells. Messenger RNA, purified from these cells, was used to generate complimentary DNA (cDNA) and combined with the growth factors' primers before amplification using Polymerase Chain Reaction (PCR). Reactions were separated by gel electrophoresis, and DNA was stained with ethidium bromide. Image analysis using the BioRad Chemidoc system was used to generate density data for each growth factor. Bands were normalized against the housekeeping gene, Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH). Our results showed that in a majority of the myofibroblast samples, as more thrombin was added, the amount of growth factors increased; thus, the myofibroblast's growth factors' mRNA expression was greatest at 1 unit/ml of thrombin. Similarly, the fibroblast expression of growth factors increased as thrombin was added; however, more thrombin did not necessarily equate to more mRNA expression for growth factors. Our results have led us to conclude that in general, thrombin increases the amount of growth factors within human corneal fibroblast and myofibroblast cells.