論　文　内　容　要　旨

An in vitro study of scarring formation mediated by human Tenon fibroblasts: Effect of Y- 27632, a Rho kinase inhibitor.
(Rho-Kinase 阻害剤、Y−27632 の術後瘢痕形成に及ぼす作用と治療への応用)
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Glaucoma filtration surgery remains the most effective procedure to produce significant intraocular pressure (IOP) reduction when medical therapy ineffectively reduced IOP. However, scar formation caused by excessive healing response on surgical site is the leading cause of failure after glaucoma filtering surgery. Fibroblast proliferation, migration, differentiation in Tenon's capsule and collagen deposition have been proposed as an important role in scar formation. Moreover, classical anti-glaucoma medication as initial and major treatment modalities for glaucoma has been hypothesized contribute to scar formation after filtering surgery. Many studies recently investigated the efficacy the Rho-associated protein kinase (ROCK) inhibitors to lower IOP with directly affect the trabecular meshwork and Schlemm's canal. In this study, we investigated the effect of ROCK inhibitor, Y-27632, in scarring formation mediated by Human Tenon Fibroblasts (HTFs) cell in direct comparison with other anti-glaucoma drugs. We used collagen gel contraction assay to compare the effect of Y-27632, other anti-glaucoma drugs (timolol and latanoprost), and TGF-β (as positive control) on collagen contraction mediated by HTFs and to evaluate the effect of Y-27632 in combination with latanoprost, timolol, and TGF-β. Western blot (WB) and immunofluorescence (IF) were examined to compare expression of factors relating scarring formation, α-SMA and vimentin, on various cell treatments. Our study demonstrated that Y-27632 inhibited collagen gel contraction, α-SMA and vimentin expressions, which was inversely promoted by latanoprost, timolol or TGF-β. Combination treatment with Y-27632 significantly suppressed contraction and upregulation of α-SMA and vimentin expression in latanoprost, timolol, and combination latanoprost/timolol groups. To confirm that the inhibition of collagen gel by Y-27632 was not correlated with apoptosis, we performed cell apoptosis assays using apoptotic/necrotic cell detection kit. HTFs treated with Y -27632 showed increase in the number of apoptotic cells, compared with control. This result implies that inhibition of collagen gel contraction by cells treated with Y -27632 was inde due to fibroblast contraction, rather than a change in the number of apoptotic cells. Given that latanoprost and timolol have been implicated to increase contraction in our previous experiment, we examined the ability of Y-27632 for blocking the contraction induced by latanoprost and timolol in HTFs cell. HTFs were stimulated with anti-glaucoma drugs for 24 hours and then treated with and without Y-27632 afterwards. Y-27632 significantly suppressed contraction effect and upregulation of α-SMA and vimentin expression in latanoprost, timolol, and latanoprost-timolol groups compare with that without Y-27632 treatment. After surgery, TGF-β is secreted to promote

wound healing, activate fibroblast, increase fibroblast proliferation and subsequently regulated fibroblast to differentiate into myofibroblast during scar formation. TGF-β also activates the MAPK pathway including ERK, p38, and JUN N-terminal kinase (JUNK) that involve in scarring formation. To examine the effect of Y-27632 in scarring formation after surgery and potential effect of Y-27632 to inhibit the effect of TGF-β, we induced the HTFs with TGF-β prior to Y-27632 to mimic TGF-β secretion which excessively secreted in post-surgical scarring. The results showed that Y-27632 could significantly block contractility effect of HTFs cultured in the presence of TGF-β as well as the marker of fibrosis. In addition, the effect of Y-27632 on phosphorylation of mitogen-activated protein kinases (MAPK) signaling activated by TGF-β was also tested in this study. Our results showed that Y-27632 significantly inhibit the activation of MAPK in TGF-β-induced HTF cells. These results suggest that ROCK inhibitor may improve the outcome after glaucoma filtering surgery with anti-scarring effect through inhibition of transdifferentiation of Tenon fibroblasts into myofibroblasts, TGF-β, and MAPK signaling pathway after surgery while scarring formation was promoted by treatment with latanoprost and timolol.