# 984 RhoA kinase-inhibition prevents myofibroblast transformation in a cell culture model of Peyronie's disease

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**Introduction & Objectives:** Peyronie's disease (PD) is a sexual debilitating disease caused by an irreversible fibrotic plaque of the penile tunica albuginea (TA). Treatment is limited to surgically restoring anatomical shape. Evidence for reliable medical treatment is lacking. This study aimed to uncover the anti-myofibroblast (MFB) properties of RhoA kinase-inhibitor (Ri) (compound Y27632) in an in vitro setting.

**Materials & Methods:** Primary cell culture was initiated from surgically obtained TA tissue from PD patients. To confirm fibroblast (FB) identity, cells were stained for vimentin (Vim) using immunofluorescence (IF). To induce MFB status, cells where stimulated with 3ng/mL TGF- β1 (TBS). Increasing doses of Y27632 where added (1-10-30mM). RT-qPCR was used to assess mRNA expression of alpha-smooth muscle actin (α-SMA) after 24h. WB was used to quantify α-SMA protein contents and IF visualized MFB differentiation by staining for α-SMA after 72h. Resazurin-based assay was performed to assess cell viability to ensure anti-MFB effect of the drugs.

**Results:** After 24h of TBS a 6-fold upregulation of α-SMA compared to non-stimulated cells could be observed. When treated with Y27632, the α-SMA mRNA expression returned to non-stimulated levels. 72h of TBS showed a 50% increase in α-SMA protein expression on WB, which was reversed to non-stimulated levels after treatment with Y27632. Using IF, TBS cells were identified as MFB (α-SMA+, Vim+) as opposed to the non-stimulated and Y27632-treated cells (α-SMA-, Vim+). The resazurin-based assay confirmed that the cell viability was not compromised when administering increasing doses of Y27632.

**Conclusions:** Transformation of fibroblasts into the contractile and extracellular matrix-producing myofibroblasts occurs after TGF- β1 stimulation. In our experiments Rho A-kinase inhibition has shown the ability to prevent the formation of myofibroblasts in TGF- β1 stimulated cells on an RNA and protein level. Y27632 could become a novel treatment option in the early treatment of PD.