Macrophages Promote Conjunctival Fibroblast Contraction And Nullify The Effect Of Anti-Scarring Agents

Abstract Number: 5618 - D0201

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Disclosure Block: Garima Sharma, None; Maryse Bailly, None; Steve Brocchini, None; Peng T. Khaw, None

Purpose: We have developed a novel in vitro model that incorporates two important aspects of fibrosis – inflammation and fibroblast-mediated tissue contraction. This study examined the effect of inflammatory stimulus provided by macrophages on the efficiency of anti-scarring drugs on fibroblast-populated collagen gel contraction.

Methods: Macrophages derived from U937 monocyte-like human cell line were co-cultured with human Tenon’s capsule fibroblasts in three-dimensional collagen gels. The gels were treated with a broad spectrum MMP inhibitor (GM6001) or Rac1 inhibitor (NSC23766). Additionally, fibroblasts were treated with different doses of Mitomycin-C (MMC) for 5 minutes, and then seeded in collagen gels at different fibroblast:macrophage ratios (1:2 or 1:5). Contraction was calculated from digital photographs using ImageJ.

Results: Broad-spectrum MMP inhibitor prevents contraction in fibroblast populated collagen gels. However, in co-culture conditions (ratio 1:2) macrophages were able to completely overcome the effect of the inhibitor (Fig. 1A). Similarly, treatment with Rac1 inhibitor for the first 24-hours also prevented fibroblast-mediated contraction for up to 4 days. The Rac1 inhibitor also successfully blocked the ability of macrophages to stimulate fibroblast-mediated gel contraction when the fibroblast:macrophage ratio was low (1:2). However, increasing the ratio to 1:5 nullified the effect of the inhibitor (Fig. 1B).

When fibroblasts were seeded alone, MMC (0.2 mg/ml) successfully inhibited contraction. With a fibroblast:macrophage ratio of 1:2, MMC at lower doses did not prevent contraction and a higher dose of MMC (1 mg/ml) was required to completely inhibit contraction. Furthermore, a higher ratio of macrophages (1:5) completely prevented the effect of MMC on contraction, even at the highest dose (Fig. 2).

Conclusions: The fibroblast sensitivity to anti-scarring treatment is significantly altered in presence of inflammation. This co-culture model for tissue contraction and inflammation in fibrosis can be used to study the effect of inflammatory stimulus on the efficacy of anti-scarring drugs. This has implications for the use and dosing of anti-scarring drugs in situations where different degrees of inflammation are present.

Layman Abstract (optional): Provide a 50-200 word description of your work that non-scientists can understand. Describe the big picture and the implications of your findings, not the study itself and the associated details.:

Macrophages can overcome the effect of high doses of MMC.