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Research Article

Activators and stimulators of soluble guanylate cyclase counteract myofibroblast differentiation of prostatic and dermal stromal cells

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ABSTRACT

Background: Fibrotic diseases encompass numerous systemic and organ-specific disorders characterized by the development and persistence of myofibroblasts. TGFβ1 is considered the key inducer of fibrosis and drives myofibroblast differentiation in cells of diverse histological origin by a pro-oxidant shift in redox homeostasis associated with decreased nitric oxide (NO)/cGMP signaling. Thus, enhancement of NO/cGMP represents a potential therapeutic strategy to target myofibroblast activation and therefore fibrosis.

Methods: Myofibroblast differentiation was induced by TGFβ1 in human primary prostatic (PrSCs) and normal dermal stromal cells (NDSCs) and monitored by α smooth muscle cell actin (SMA) and IGF binding protein 3 (IGFBP3) mRNA and protein levels. The potential of enhanced cGMP production by the sGC stimulator BAY 41-2272 or the sGC activator BAY 60-2770 to inhibit and revert myofibroblast differentiation in vitro was analyzed. Moreover, potential synergisms of BAY 41-2272 or BAY 60-2770 and inhibition of cGMP degradation by the PDE5 inhibitor vardenafil were investigated.

Results: BAY 41-2272 and BAY 60-2770 at doses of 30 μM significantly inhibited induction of SMA and IGFBP3 levels in PrSCs and reduced myofibroblast marker levels in TGFβ1-predifferentiated cells. At lower concentrations (3 and 10 μM) only BAY 41-2272 but not BAY 60-2770 significantly inhibited and reverted myofibroblast differentiation. In NDSCs both substances significantly inhibited differentiation at all concentrations tested. Attenuation of SMA expression was more pronounced in NDSCs whereas reduction of IGFBP3 levels by BAY 41-2272 appeared more efficient in PrSCs. Moreover, administration of BAY 41-2272 or BAY 60-2770 enhanced the efficiency of the PDE5 inhibitor vardenafil to inhibit and revert myofibroblast differentiation in vitro.

Conclusions: Increase of cGMP by sGC stimulation/activation significantly inhibited and reverted myofibroblast differentiation. This effect was even more pronounced when a combination treatment with a PDE5 inhibitor was applied. Thus, enhancement of NO/cGMP-signaling by sGC stimulation/activation is a promising strategy for the treatment of fibrotic diseases. Whereas, in NDSCs BAY 60-2770 and BAY 41-2272 exerted similar effects on myofibroblast differentiation, higher potency of BAY 41-2272 was observed in PrSCs, indicating phenotypical differences between fibroblasts form different organs that should be taken into account in the search for antifibrotic therapies.

1. Introduction

Dysregulation of the wound healing response can result in fibrosis characterized by excessive accumulation of extracellular matrix resulting in chronic scaring of affected organs [1,2]. Fibrotic diseases include multisystemic disorders such as systemic sclerosis, chronic graft versus host disease or nephrogenic systemic

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fibrosis and organ-specific diseases like cardiac fibrosis, idiopathic pulmonary fibrosis, intestinal fibrosis, liver cirrhosis or benign prostatic hyperplasia (BPH) [1–5]. Additionally, many disorders associated with prominent tissue remodeling have a significant fibrotic component, including asthma, atherosclerosis and the reactive stromal response to solid tumors, such as breast, liver and prostate cancer [3,6,7].

Fibrogenesis in most organs and tissues is characterized by the development and persistence of large numbers of myofibroblasts [1,5], a specialized cell type that combines the ECM-producing characteristics of fibroblasts with the cytoskeletal and contractile properties of smooth muscle cells [4]. Whereas in normal wound healing upon completion of re-epithelialization myofibroblasts undergo apoptosis, persistent myofibroblast activation in fibrotic tissue leads to excessive ECM deposition, altered growth factor signaling and consequently to cellular proliferation, progressive remodeling and destruction of normal tissue architecture, organ dysfunction and failure [3,5]. Thus, the myofibroblast represents the main effector cell of fibrosis and thereby a major therapeutic target.

Myofibroblasts predominantly originate from the differentiation of local tissue fibroblasts [8]. TGFβ1 is considered to be the key inducer of fibrosis and drives myofibroblast differentiation in cells of diverse histological origin. Consistently, elevated TGFβ1 levels and signaling are observed in many fibrotic disorders [3]. TGFβ-induced myofibroblast differentiation is driven by a pro-oxidant shift in redox homeostasis due to elevated NADPH oxidase 4-derived hydrogen peroxide and a concomitant decrease in nitric oxide (NO)/cGMP signaling [2,3]. Thus, enhancement of NO/cGMP signaling either by enhancing cGMP production or inhibiting cGMP degradation represents a potential therapeutic strategy to target myofibroblast activation and therefore fibrosis. Consistent with this concept, we previously demonstrated that inhibition of the cGMP degrading phosphodiesterase type 5 (PDE5), significantly inhibited and reverted TGFβ1-induced myofibroblast differentiation of prostatic fibroblasts [9,10]. Additionally, PDE5 inhibition prevented myofibroblast differentiation in cardiac fibroblasts and in fibroblasts form Peyronie´s disease plaques in vitro and counteracted fibrosis in rodent models of cardiac fibrosis and Peyronie's disease [11–13].

In addition, we and others previously demonstrated that enhancement of NO/cGMP signaling by soluble NO donors inhibited or reverted myofibroblasts differentiation of prostatic and dermal fibroblasts or hepatic stellate cells in vitro and attenuated liver fibrosis in cirrhotic rats [9,14–16]. Since NO activates soluble guanylate cyclase (sGC), increasing sGC activity via heme-dependent sGC stimulators or heme-independent sGC activators represents an alternative approach to enhance NO/cGMP signaling. In deed the sGC stimulator BAY 41-2272 inhibited or reverted myofibroblast differentiation of dermal and cardiac fibroblasts in vitro and limited disease progression or reduced established fibrosis in models of renal, cardiac and dermal fibrosis [17–20]. Similarly, the sGC activator BAY 60-2770 attenuated liver fibrosis in rat models [21].

However, fibroblast phenotypes could be different and therefore antifibrotic properties of sGC stimulators and sGC activators may differ and may be tissue-specific. Therefore, in the present study, the potential of sGC stimulation by BAY 41-2272 and sGC activation by BAY 60-2770 to inhibit and revert myofibroblast differentiation of primary prostatic as well as dermal fibroblasts was compared using the well-established myofibroblast differentiation/fibrosis markers α-smooth muscle cell actin (SMA; ACTA2) and insulin-like growth factor binding protein 3 (IGFBP3) [4,5,17,22–24]. Moreover, potential synergisms of sGC stimulation or activation and concomitant PDE5 inhibition by vardenafil were investigated.

2. Materials and methods

2.1. Culture of human primary cells and prostatic organoids, and myofibroblast differentiation

Human primary prostatic stromal cells (PrSC) were established from regularly supplied prostate tissue as described previously [25] and cultured in stromal cell growth medium (Quantum 333, PAA Laboratories). Human normal dermal stromal cells (NDSC) were established by the same protocol using skin removed from the abdomen in the course of plastic surgery from patients after written informed consent.

Fibroblast-to-myofibroblast differentiation was induced by 1 ng/ml TGFβ1 (R & D Systems) while control cells were incubated with 1 ng/ml bFGF (Sigma Aldrich) in RPMI1640 (PAA Laboratories) supplemented with 1% charcoal-treated bovine calf serum (Hyclone Laboratories) and antibiotics for 72 h as described [9]. To investigate the potential of BAY 41-2272 (Bayer Healthcare) or BAY 60-2770 (Bayer Healthcare) alone or in combination with vardenafil (Bayer Healthcare) to inhibit differentiation, stromal cells were pretreated with the respective substances at concentrations indicated prior to addition of TGFβ1.

For differentiation reversal studies, cells were differentiated with TGFβ1 for 72 h and subsequently stimulated for additional 72 h in the presence of TGFβ1 with BAY 41-2272 or BAY 60-2770 alone or in combination with vardenafil at indicated concentrations.

Human prostatic organoids were established as described previously [10] and cultured in serum-free RPMI1640 containing antibiotics supplemented with 10 μM BAY 41-2272, 10 μM BAY 60-2770 or mock control for 7 days prior to RNA isolation.

Each experiment was repeated at least 3 times with primary cells of passages 2–4 from different donors.

2.2. Quantitative real-time PCR

mRNA extraction, cDNA synthesis and quantitative PCR (qPCR) were performed as described elsewhere [9,26]. Primer sequences are given in Table 1. For PrSC and NDSC experiments cDNA concentrations were normalized by the internal standard hydroxymethylbilane synthase (HMBS), for prostatic organoids cDNA was normalized to the arithmetic mean of the ct-values of HMBS, eukaryotic translation elongation factor 1 alpha 1 (EEF1A1) and beta actin (ACTB).

2.3. Western blot analysis

Fibroblast-to-myofibroblast differentiation was monitored at protein levels of SMA and IGFBP3 by Western blot analysis as described previously [25] using goat anti-human IGFBP3 (at a dilution of 1:1000, R&D Systems), mouse anti-human α-SMA

Table 1
Primer sequences.

Gene	Primer sequences	
	Sense	Antisense
ACTA2 (SMA)	5-tacaatgagcttcgtgttgc	5-cgtccagaggcatagagaga
ACTB	5-gacgacatggagaaaatctg	5-acatgatctgggtcatcttct
CNN1	5-ggtgaacgtgggagtgaagt	5-ggtccagaggctggtctgt
EEF1A1	5-cacacggctcacattgca	5-cacgaacagcaaagcgacc
HMBS	5-ccaggacatcttggatctgg	5-atggtagcctgcatggtctc
IGFBP3	5-caagcgggagacgaatatg	5-ttatccacacaccagcagaa
NOX4	5-tggcaagagaacagacctga	5-tgggtccacaacagaaaaca
PLN	5-acagctgccaaggctaccta	5-gcttttgacgtgcttgttga