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Phosphodiesterase-5/5-HT2B dual inhibitors abrogate completely fibrotic potential of human renal fibroblasts isolated from renal allograft rejection patients

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Objectives: Despite improvements in immunosuppressive therapy, long-term allograft survival after kidney transplantation remains as low as 50%. The primary cause of chronic allograft nephropathy is "interstitial fibrosis and tubular atrophy" (IF/TA). Serotonin (5-HT; 5-Hydroxytryptamine) produces extracellular matrix proteins in presence of TGF-β1 dependent manner. TGF-β1 activates resident fibroblasts, trans-differentiate into myo-fibroblasts, which is the hallmark of the pathogenesis of fibrosis. Here we evaluate the anti-fibrotic efficacy of phosphodiesterase-5 (PDE-5) inhibitor, Sildenafil, and 5-HT_{2B} inhibitor, SB204741 in combination, on renal fibroblast isolated from renal allograft rejection patients.

Methods: Renal fibroblasts were isolated from nephrectomy of control (n=3) and renal allograft rejection patients (n=3). Fibroblasts were incubated with TGF-β1 (10 ng/ml) for 1 hour, later with TGF-β1 (10 ng/ml) and [Sildenafil (10μM) plus SB204741 (1μM)] for 24 hours (post-treatment strategy). In the pre-treatment strategy, fibroblasts were pre-treated with [Sildenafil (10 μM) plus SB204741 (1 μM)] for 1 hour and later with only TGF-β1 (10 ng/ml) for 24 hours. Similar strategies were followed for individual treatments of inhibitors. Real-time qPCR for pro-fibrotic genes, COL1A1, ACTA2, CTGF, and fibronectin1, and anti-fibrotic genes, TIMP1, MMP2 was performed. Type 1 collagen and α-SMA proteins were examined by western blotting.

Results: In TGF-β1 stimulated fibroblasts, significant up-regulation of pro-fibrotic gene expression was observed, significantly reducing co-culture with PDE-5 plus 5-HT_{2B} inhibitors. The ratio of anti-fibrotic genes (MMP2/TIMP1) was restored significantly. The expression of type 1 collagen was decreased significantly. Furthermore, near-complete amelioration of ACTA2, as well as α-SMA protein, was observed significantly (Table 1).

Conclusions: Dual inhibition combination of PDE-5 plus 5-HT_{2B} inhibitors lead to near-complete attenuation of conversion of resident fibroblasts to myo-fibroblasts and thus may have the prospective for treatment of fibrosis of renal allograft rejection patients.

Table 1

Genes	TGF-β1 treatment (Fold change in comparison to media+ cells only)	TGF-β1+ Sildenafil + SB204741 (Fold change in comparison to TGF-β1 stimulation)
Post-treatment strategy		
*COL1A1	Reference (4.2 fold increase)	(1.4 fold decrease)
*ACTA2	Reference (4.5 fold increase)	(2.0 fold decrease)
*CTGF	Reference (7.9 fold increase)	(3.3 fold decrease)
*FN1	Reference (5.2 fold increase)	(1.3 fold decrease)
*MMP2	Reference (0.4 fold decrease)	(0.5 fold increase)
*TIMP1	Reference (3.5 fold increase)	(1.5 fold decrease)
* MMP2/TIMP1	Reference (0.3 fold decrease)	(0.5 fold increase)
*Type 1 collagen protein	Reference (4.4 fold increase)	(1.8 fold decrease)
*α-SMA protein	Reference (2.5 fold increase)	(1.3 fold decrease)
Pre-treatment strategy		
*COL1A1	Reference (5.2 fold increase)	(3.4 fold decrease)
*ACTA2	Reference (4.5 fold increase)	(3.8 fold decrease)
*CTGF	Reference (7.9 fold increase)	(6.5 fold decrease)
*FN1	Reference (5.5 fold increase)	(3.2 fold decrease)
*MMP2	Reference (0.4 fold decrease)	(1.2 fold increase)
*TIMP1	Reference (3.2 fold increase)	(2.6 fold decrease)
* MMP2/TIMP1	Reference (0.3 fold decrease)	(1.3 fold increase)
*Type 1 collagen protein	Reference (3.5 fold increase)	(2.1 fold decrease)
*α-SMA protein	Reference (2.9 fold increase)	(2.4 fold decrease)
* Values marked with asterix indicate those attaining statistical significance ($p<0.05$)		