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Phosphodiesterase-5/5-HT2B inhibitors in combination almost completely abrogate fibrotic potential of human peritoneal fibroblasts isolated from CAPD patients

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Objectives: Peritoneal fibrosis (PF) leads to ultrafiltration failure (UF) in continuous ambulatory peritoneal dialysis (CAPD) patients. Serotonin (5-HT; 5-Hydroxytryptamine) produces extracellular matrix (ECM) proteins in human peritoneal fibroblasts (HPFB) in transforming growth factor beta 1 (TGF-β1) dependent manner which activates resident fibroblasts, trans-differentiate into myo-fibroblasts (MFBs), which are characterized by increased α-smooth muscle actin (α-SMA) expression and ECM proteins production. Here we evaluate the anti-fibrotic efficacy of phosphodiesterase-5 (PDE-5) inhibitor, Sildenafil, and 5-HT_{2B} inhibitor, SB204741, in combination on HPFB.

Methods: HPFB isolated from parietal peritoneum biopsy (PB) excised during laparotomy of control (n=8)/CAPD patients (n=6), incubated overnight in dispase (2.4 U/mL)/37°C. HPFB were incubated with TGF-β1 (10 ng/ml)/1 hour, later with TGF-β1 (10 ng/ml)/[Sildenafil (10μM)+SB204741 (1μM)] for 24 hours (post-treatment strategy). In pre-treatment strategy, HPFB were pre-treated with [Sildenafil (10 μM) +SB204741 (1 μM)]/1 hour and later with only TGF-β1 (10 ng/ml)/24 hours. Similar strategies were followed for individual treatments of inhibitors. Real time qPCR for pro-fibrotic genes, COL1A1, COL1A2, ACTA2, CTGF, FN1 and anti-fibrotic genes, TIMP1, MMP2 was performed. GAPDH was housekeeping gene. Immunoblotting was performed for expression of α-SMA and type 1 collagen. The results are represented as mean±SEM. All the groups were evaluated for the level of significance by using Wilcoxon matched pairs signed rank test. Significance was set at $p \leq 0.05$.

Results: In TGF-β1 stimulated HPFB, significant up-regulation of pro-fibrotic genes expression was observed, which significantly reduced on co-culture with PDE-5 plus 5-HT_{2B} inhibitors. Ratio of anti-fibrotic genes (MMP2/TIMP1) was restored significantly. 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 MFBs and thus may have the prospective for treatment of fibrosis of peritoneum in CAPD patients.

Table 1

Table 1	**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 (5.3 fold increase)	(1.5 fold decrease)
**COL1A2*	Reference (4.1 fold increase)	(1.1 fold decrease)
**ACTA2*	Reference (4.7 fold increase)	(2.2 fold decrease)
**CTGF*	Reference (8.9 fold increase)	(4.3 fold decrease)
**FN1*	Reference (5.4 fold increase)	(1.1 fold decrease)
**MMP2*	Reference (0.4 fold decrease)	(0.6 fold increase)
**TIMP1*	Reference (3.1 fold increase)	(1.3 fold decrease)
* MMP2/TIMP1	Reference (0.3 fold decrease)	(0.5 fold increase)
*Type 1 collagen protein	Reference (3.4 fold increase)	(1.5 fold decrease)
*α-SMA protein	Reference (2.8 fold increase)	(1.2 fold decrease)
Pre-treatment strategy		
**COL1A1*	Reference (5.3 fold increase)	(3.2 fold decrease)
**COL1A2*	Reference (4.1 fold increase)	(2.2 fold decrease)
**ACTA2*	Reference (4.7 fold increase)	(3.9 fold decrease)
**CTGF*	Reference (8.9 fold increase)	(6.6 fold decrease)
**FN1*	Reference (5.4 fold increase)	(3.0 fold decrease)
**MMP2*	Reference (0.4 fold decrease)	(1.3 fold increase)
**TIMP1*	Reference (3.1 fold increase)	(2.5 fold decrease)
* MMP2/TIMP1	Reference (0.3 fold decrease)	(1.1 fold increase)
*Type 1 collagen protein	Reference (3.4 fold increase)	(2.0 fold decrease)
*α-SMA protein	Reference (2.8 fold increase)	(2.3 fold decrease)
* Values marked with asterix indicate those attaining statistical significance ($p<0.05$)		