KSN 2021
FULLY VIRTUAL MEETING
September 02 (Thu) - 05 (Sun)

Oral Communication Abstract
Presentation No. OC8-02 (Abstract Submission No. 2493)

Oral Communications 8 Sep. 4 (Sat), 08:30-10:30

Phosphodiesterase-5 inhibitor/SB204741 in combination almost completely ameliorate fibrotic potential of human peritoneal fibroblasts isolated from CAPD patients

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Objectives: Peritoneal fibrosis (PF) leads to ultrafiltration failure in patients during long-term continuous ambulatory peritoneal dialysis (CAPD). Serotonin (5-HT; 5-Hydroxytryptamine) produces extracellular matrix (ECM) proteins in transforming growth factor beta 1 (TGF-β1) dependent manner. Here we evaluate the anti-fibrotic efficacy of phosphodiesterase-5 (PDE-5) inhibitor, Sildenafil, and 5-HT_{2B} inhibitor, SB204741, in combination on human peritoneal fibroblasts (HPFBs) isolated from parietal peritoneum biopsy of CAPD patients.

Methods: PB was excised when patients were undergoing catheter removal and controls for elective cholecystectomy. HPFBs 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 pre-treatment strategy, HPFBs 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, collagen type I alpha 1 chain (COL1A1), collagen type I alpha 2 chain (COL1A2), smooth muscle alpha (α)-2 actin (ACTA2), connective tissue growth factor (CTGF) and fibronectin1 (FN1) and anti-fibrotic genes, tissue inhibitor of metalloproteinases1 (TIMP1), matrix metalloproteinase2 (MMP2) was performed. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was housekeeping gene. Type 1 collagen and α-SMA proteins were examined by immunoblotting

Results: In TGF-β1 stimulated HPFBs, 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 abrogation of conversion of resident fibroblasts to MFBs and thus may have the prospective for treatment of fibrosis of peritoneum in CAPD patients.

Fold changes in pro-fibrotic, anti-fibrotic & protein

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 ($p<0.05$)		