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Terguride and SB204741 reduce fibrotic potential of human peritoneal fibroblasts by targeting STAT3 pathway in patients receiving continuous ambulatory peritoneal dialysis

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Objectives: Peritoneal fibrosis (PF) results in ultrafiltration failure in patients on long term continuous ambulatory peritoneal dialysis (CAPD). 5-hydroxytryptamine (5-HT; serotonin) induces extracellular matrix (ECM) synthesis in a transforming growth factor beta 1 (TGF-β1) dependent manner. We evaluated anti-fibrotic role of inhibitors of 5-HT_2(Terguride) and 5-HT_{2B}(SB204741) in in human peritoneal fibroblasts (HPFBs) isolated from peritoneum of CAPD patients .

Methods: Biopsy from parietal peritoneum (PB) of control patients (n=8) and CAPD patients (n=6) excised during laparotomy was incubated overnight in dispase (2.4 U/mL)/37°C.In post-treatment strategy, HPFBs isolated from CAPD patients and controls,were incubated with 5-HT (1μM)/TGF-β1 (10ng/ml) for 1 hour and later with 5-HT (1μM)/TGF-β1 (10ng/ml) and terguride or SB204741 (1μM, each) for 24 hours. , cells were pre-treated with terguride or SB204741 (1μM, each) for 1 hour and later with only 5-HT (1μM)/TGF-β1 (10ng/ml) for 24 hours (pre-treatment strategy).Real time quantitative PCR for pro-fibrotic (*TGFB1, COL1A1, COL1A2, ACTA2, CTGF*and *FN1*) and anti-fibrotic genes (*MMP2/TIMP1*) expression was performed. Type I collagen and α-SMA, phosphorylation status of Smad-3, ERK1/2, Src and STAT-3 was examined by western blotting.

Results: In 5-HT and TGF-β1stimulated HPFB, upregulated expression of *COL1A1, COL1A2, ACTA2, CTGF* and *FN1*($p<0.05$) mRNA at 24 hr was observed. Co-culture of HPFB with 5-$HT_2$and 5-HT_{2B}receptor antagonistssignificantly reduced pro-fibrotic genes expression ($p<0.05$) in both the strategies. Effect on anti-fibrotic genes mRNA in both the strategies was not affected. 5-HT dose-dependently increased the mRNA levels of *TGF-B1*. Terguride and SB204741 mitigated alpha smooth muscle actin protein levels (figure 1) but they did not influenced Smad3 phosphorylation (canonical pathway, figure 2) rather they significantly reduced STAT3 phosphorylation (non-canonical pathway, figure 3) ($p<0.05$). However no effect on Src phosphorylation (figure 4) was observed.

Conclusions: TGF-β1 mediated $ERK_{1/2}$/STAT3 pathways have been implicated in pro-fibrotic genes regulation in PF and 5-HT receptor antagonists attenuate 5-HT/TGF-β1 mediated pro-fibrotic potential of HPFBs.