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5-HT2 AND 5-HT2B RECEPTOR ANTAGONISM REDUCE PERITONEAL FIBROSIS BY TARGETING NON-CANONICAL PATHWAYS IN CAPD PATIENTS

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Objectives: To evaluate anti-fibrotic role of inhibitors of 5-HT_2 and 5-HT_{2B} (Terguride and SB204741), respectively in human peritoneal fibroblasts (HPFB) 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, cells were incubated with 5-HT(1µM) for 1 hr and later with 5-HT (1µM) and terguride or SB204741 (1µM, each) for 24 hr. In pre-treatment strategy, cells were pre-treated with terguride or SB204741 (1µM, each)for 1 hr and later with only 5-HT (1µM) for 24 hr.HPFB were also incubated with TGF-β1 (10ng/ml) and 5-HT inhibitors similar to the above strategies. Real time quantitative PCR for pro-fibrotic (*TGF-B1, 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, Srcand STAT-3 was examined by immunoblotting.

Results: In 5-HT and TGF-β1stimulated HPFB, upregulated expression of *COL1A1, COL1A2, ACTA2, CTGF* and *FN1* ($p<0.05$) mRNA at 24 hrwas observed. Co-culture of HPFB with 5-HT_2 and 5-HT_{2B} receptor antagonists significantly 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. Pre-treatment with both 5-HT inhibitors decreased the production of type 1 collagen and α-SMA significantly ($p<0.05$). 5-HT dose-dependently increased the mRNA levels of *TGF-B1*. Terguride and SB204741 did not influence Smad-3 phosphorylation (canonical pathway, figure 1) rather they significantly reduced ERK1/2 and STAT3 phosphorylation (non-canonical pathway, figure 2 and 3 respectively) ($p<0.05$). However no effect on Src phosphorylation (figure 4) was observed.

Conclusions: TGF-β1 mediated non-canonical pathways, ERK1/2 and STAT3 have been implicated in regulation of pro-fibrotic genes and in the development of fibrosis. 5-HT receptor antagonists might reduce fibrosis via suppression of TGF-β1 mediated non-canonical pathways.