5-HT₂ and 5-HT_{2B} inhibitors mitigate fibrotic phenotype of peritoneal fibroblasts via non-canonical signaling in patients on continuous ambulatory peritoneal dialysis

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Background

Peritoneal fibrosis is a major cause of ultrafiltration failure in continuous ambulatory peritoneal dialysis (CAPD) patients. 5-hydroxytryptamine (5-HT) induces extracellular matrix synthesis in peritoneal fibroblasts in Transforming growth factor beta 1 (TGF β 1) dependent manner. We aimed to evaluate antifibrotic role of 5-HT₂ and 5-HT_{2B} inhibitors Terguride and SB 204741 respectively in human peritoneal fibroblasts (HPFB) isolated from omentum and parietal peritoneum of CAPD patients.

Methods

Omentum biopsy (OB) of control patients (n=6) undergone elective abdominal surgery and CAPD patients (n=4) was washed with sterile PBS and incubated in 0.125% trypsin/0.01% EDTA twice for 20 minutes followed by two times for 40 minutes at 37°C. Biopsy from parietal peritoneum PB excised during laparotomy incubated overnight in dispase (2.4 U/mL)/37°C. After centrifugation pellet obtained from OB and PB incubated in complete DMEM gave HPFB within 2 weeks. In first strategy HPFB from OB and PB were incubated with (5-HT-1 μ M) for 1 hr and later with (5-HT-1 μ M) and (Terguride, SB 204741-1 μ M each) for 24 hr. In second strategy HPFB from OB and PB were pre-treated with (Terguride, SB 204741-1 μ M each) for 1 hr and later with only (5-HT-1 μ M) for 24 hr. Real time qPCR for pro-fibrotic (TGF β 1, Col1a1, Col1a2, ACTA2, CTGF and FN1) and anti-fibrotic genes (MMP2/TIMP1) expression was performed. Type I collagen and α SMA phosphorylation status of Smad3 and ERK1/2 was examined by immunoblotting.

Results

In 5-HT stimulated HPFB upregulated expression of profibrotic genes (p<0.05) mRNA at 24 hr was observed. Co-culture of HPFB with 5-HT₂ and 5-HT_{2B} receptor antagonists Terguride and SB 204741 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 Terguride and SB 204741 decreased the production of type 1 collagen and α SMA significantly (p<0.05). 5-HT dose dependently increased the mRNA levels of TGF β 1. Terguride and SB 204741 did not influence Smad3 phosphorylation (canonical pathway) rather they significantly reduced ERK1/2 phosphorylation (noncanonical pathway) p<0.05.

Conclusion

5-HT₂ and 5-HT_{2B} receptor antagonists reduce profibrotic mRNA expression in 5-HT stimulated HPFB by suppressing TGF β 1 mediated noncanonical pathway.