Title	Induction of interleukin-6 secretion and matrix protein synthesis in human peritoneal mesothelial cells (HPMCs) by Pseudomonas aeruginosa exotoxin pyocyanin
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Basic Research on Biocompatibility, Immunology, Inflammation, and Fibrosis

Fibrosis-Related Growth Factors in Peritoneal Dialysate During Peritonitis

Objective: Peritonitis (P) remains an important cause of technique failure in peritoneal dialysis (PD). Studying the serial levels of fibrosis-related growth factors in spent peritoneal dialysate during P can give insight into the mechanisms of peritoneal fibrosis. This study aimed to examine the profile of these growth factors in relation to microbiologic and clinical characteristics. *Methods:* 31 episodes of bacterial P (14 men, 17 women; mean duration of PD 42.9±33.0 months) were studied. Dialysate samples from 20 patients who were P-free for >6 months served as controls. 20 mL of dialysate at each time point was collected, centrifuged, filtered sterilized, and stored at –70°C until analysis. TGF-β1, HGF, BMP-7, and VEGF were assayed using commercial ELISAs. Cultured human peritoneal mesothelial cells were incubated with dialysate specimens for up to 24 hours, and the cell morphology was studied by phase contrast microscopy. *Results:* Gram +ve and gram –ve organisms accounted for 15 and 16 P episodes respectively, and the species included *Streptococcus* (*n*=10), *Staphylococcus* (*n*=5), *E. coli* (*n*=6), *Pseudomonas* (*n*=4), and *Klebsiella* (*n*=6). Maximum induction of TGF-β1 and VEGF occurred at the onset of P (55.9±11.1 and 13.9±3.6 pg/mL respectively for gram +ve infection, and 35.8±12.1 and 7.2±4.8 pg/mL respectively for gram –ve infection; $p<0.001$ for gram +ve vs gram –ve). In contrast, maximum HGF levels were observed 2 days later (1074.0±167.0 vs 390.5±26.5 for gram +ve versus gram –ve, $p<0.01$). TGF-β1, VEGF, and HGF remained elevated 6 weeks later, despite clinical resolution of P ($p<0.05$ compared to controls). BMP-7 was not detectable in both noninfected and infected dialysate samples. Exposure of cultured mesothelial cells to P dialysate samples resulted in denudation of the monolayer and features of mesothelial-to-mesenchymal transdifferentiation. *Conclusions:* Our data demonstrated sequential induction of fibrosis-related growth factors during P complicating PD, and prolonged induction despite apparent clinical resolution of P after antibiotic treatment. The data also suggested more severe pathophysiologic changes during gram +ve P.

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Angiotensin II Induces Fibronectin Expression in Rat Peritoneal Mesothelial Cells via ERK 1/2

Background: The rennin–angiotensin system has been implicated in the pathogenesis of fibrosis in various organs. However, its involvement in peritoneal fibrosis is unclear. Peritoneal mesothelial cells play a major role in peritoneal fibrosis by producing extracellular matrix (ECM) consisting of collagen, fibronectin, laminin, proteoglycans, and cytokines (TGF-β, CTGF, and VEGF). However, the effect of angiotensin II (Ang II) on ECM and cytokines expression and signal transduction pathways in peritoneal mesothelial cells is poorly understood.

Methods: We measured the concentration of Ang II in peritoneal dialysis effluent and supernatant of cultured rat mesothelial cells by radioimmunoassay. Then we examined the effect of Ang II upon TGF-β, CTGF, and fibronectin production in rat mesothelial cells by RT-PCR and Western blot. Furthermore we studied the intracellular signaling pathways involved.

Results: We found there is low level of Ang II existing in peritoneal dialysis effluent and supernatant of rat mesothelial cells cultured by high glucose (1.5%, 2.5%), which suggested high glucose could stimulate the secretion of Ang II in the mesothelial cells. RT-PCR and Western blotting showed that 10 mmol/L Ang II increased TGF-β, CTGF, and fibronectin mRNA expression followed by their protein expression. AT1 receptor antagonist losartan and ERK inhibitor PD98059 can inhibit fibronectin protein enhanced by Ang II. Ang II-induced fibronectin expression was mediated by the activation of extracellular signal-regulated kinase ½ (ERK 1/2).

Conclusion: These results indicate Ang II can induced TGF-β, CTGF, and fibronectin expression in rat peritoneal mesothelial cells, and suggested it may play an important role in the peritoneal fibrosis. AT1 receptor blockers and ERK 1/2 blockers may be useful in treating and preventing peritoneal fibrosis during long-term peritoneal dialysis.

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Effects of Overexpression Smad7 on the Expression of AQP-1,3 in a Rat Peritoneal Fibrosis Model

Objectives: To investigate the effects of overexpression Smad7 on the expression of peritoneal aquaporins-(AQP-)1, -3 in a rat peritoneal fibrosis (PF) model. *Methods:* PF rat was established by daily intraperitoneal injection of 4.25% peritoneal dialysis solution for 4 weeks and intraperitoneal injection of LPS on days 1, 3, 5, 7. 40 male Sprague–Dawley rats were randomly divided into 4 groups: group N: normal control; group M: PF rat and received no treatment; group V: PF rat and received twice intraperitoneal injection of control empty vectors at days 0 and 14; group T: PF rat and received twice intraperitoneal injection of the pTRE-m2Smad7/Tet-on plasmids/Optison™ using an ultrasound at days 0 and 14. Doxycycline was added in the daily drinking water to induce the transgene expression of Smad7. The expression of AQP-1 and AQP-3 were examined by immunofluorescence, Western-blot, and RT-PCR. *Results:* The Smad7 transgene expression, peritoneal function (UF and MGT) were measured, and peritoneal AQP-1, AQP-3 mRNA and protein expression were also detected. The ultrasound treatment with Optison™ largely increased the transfection rate of Smad7 transgene expression, and this was mainly found in peritoneal mesothelium cells. Compared with normal rats, the phosphorylation of smad2/3 protein was upregulated 3.5-fold by LPS and PDS. However, treatment with inducible Smad7 resulted in a 54% decreased smad2/3 phosphorylation. Compared with normal rats, ultrafiltration (UF) was reduced significantly by LPS and PDS. In contrast, Smad7 treatment resulted in increase of UF. However, compared with PF rats, Smad7 treatment has no effects on the expression of AQP-1 and AQP-3. *Conclusions:* Overexpression of Smad7 significantly reduced the phosphorylation of Smad2/3. Blockade of TGF-β/Smad signaling by overexpression Smad7 increased UF, improved peritoneal function. However, Smad7 treatment has no effects on the expression of AQP-1 and AQP-3 in peritoneum.

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Induction of Interleukin-6 Secretion and Matrix Protein Synthesis in Human Peritoneal Mesothelial Cells (HPMCs) by *Pseudomonas aeruginosa* Exotoxin Pyocyanin

Objectives: Peritonitis (P) due to *Pseudomonas aeruginosa* is a serious complication in patients undergoing peritoneal dialysis (PD). *P. aeruginosa* has evolved immuno-evasive strategies against host immunity. Inflammatory processes are induced through the generation of toxic secondary metabolites, know as phenazines, the most abundant being pyocyanin. The impact of pyocyanin in peritoneal inflammation has not been investigated. *Methods:* Spent PD fluid samples were obtained serially from patients with P due to *P. aeruginosa* (*n*=5). HPMC were isolated from omental specimens by enzymatic disaggregation and maintained in Medium 199 supplemented with 10% FCS. All experiments were performed on cells of the second passage that had been growth arrested for 72 hours. HPMC were cultured with pyocyanin (2.5 μg/mL, the optimum non-cytotoxic dose) in the absence or presence of interleukin (IL)-1β, IL-6, or TNF-α (1 ng/mL for all) for periods up to 24 hours, the culture supernatant decanted, and mRNA or protein isolated for RT-PCR or Western blot analysis respectively. IL-6 secretion was assessed by commercial ELISA. *Results:* Spent PD fluid from *P. aeruginosa* P induced IL-6, fibronectin, and collagen type I mRNA in HPMC. This was accompanied by increased IL-6 secretion, PKC-α activation, and deposition of fibronectin and collagen type I into the extracellular milieu. This induction effect was observed in PD fluid obtained 42 days after onset of infection, despite complete clinical resolution of P symptoms. In separate studies, pyocyanin alone did not affect IL-6 secretion, but it acted synergistically with IL-6, IL-1β, and TNF-α to increase IL-6 secretion (0.11±0.01 vs 5.01±1.0 ng/mL for IL-6 alone vs IL-6 and pyocyanin, maximum effect at 3 hours; 88.1±6.2 vs 128±5.4 ng/mL for IL-1β vs IL-1β and pyocyanin, maximum at 12 hours; 22.3±6.4 vs 35.8±6.7 for TNF-α vs TNF-α and pyocyanin, maximum at 12 hours; $p<0.05$ for all). Increased IL-6 secretion was accompanied by an increase in fibronectin synthesis. *Conclusions:* Our data suggest that pyocyanin acts synergistically with inflammatory mediators to enhance the inflammatory and fibrotic response in *P. aeruginosa* P complicating PD.

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