PO2451

Complement C5a Receptor in Macrophage-Mediated Renal Inflammation and Fibrosis in Lupus Nephritis
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Background: Lupus nephritis (LN) is caused by autoimmune responses and is a significant driver of end-stage renal disease in systemic lupus erythematosus patients. Complement activation, pro-inflammatory cytokine production, and the influx of macrophages have all been implicated in LN pathogenesis. The anaphylatoxin complement 5a (C5a) receptor 1 (C5ar1) is a major driver of the pro-inflammatory functions of complement activation. We examined C5ar1's expression in kidney in lupus nephritides and investigated its role in controlling pro-fibrotic functions of macrophages.

Methods: C5ar1 expression, infiltrating immune cells, and fibrosis were examined by immunohistochemistry in LN patient kidney biopsies. M1 and M2 macrophages derived from human peripheral blood monocytes were used in in vitro assays to examine the effect of C5a stimulation and avacopan, a specific C5ar1 inhibitor, on the secretion of cytokines and other factors.

Results: In LN kidney biopsies, large numbers of macrophages, identified by CD68 staining, were observed in areas with severe fibrosis, and expressed C5ar1. In addition, C5ar1 was detected on distal tubules in biopsies of both normal and lupus nephritis kidneys. C5a increased the production of inflammatory cytokines TNFα and IL-6 from both M1 and M2 macrophages in vitro. Chemokines (MCP-3, MIP-1α, MIP-1b and MIP-3α), matrix metalloproteinases (MMP3 and MMP8), and pro-fibrotic growth factors (fibroblast activation protein, platelet-derived growth factor-A) were strongly increased in M2 macrophages with C5a stimulation, and these increases were blocked by the C5ar1 inhibitor avacopan.

Conclusions: C5ar1 activation induced macrophage secretion of factors that are known to cause inflammation, fibroblast activation and tissue fibrosis, and thus may contribute to LN disease progression. Inhibiting C5ar1 activity with avacopan blocks these pathological changes, and may provide therapeutic benefit to LN patients.

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PO2452

Remdesivir Inhibits Tubulointerstitial Fibrosis in Obstructed Kidneys
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Background: Kidney impairment is observed in patients with COVID-19. The effect of anti-COVID-19 agent remdesivir on kidneys is currently unknown. We aimed to determine the effect of remdesivir on renal fibrosis and its downstream mechanisms.

Methods: Remdesivir and its active nucleoside metabolite GS-441524 were used to treat TGF-β1-stimulated renal fibroblasts (NRK-49F) and human renal epithelial (HK2) cells. Vehicle or remdesivir were given by intravenous injection or renal injection through the left ureter in unilateral ureteral obstruction (UUO) mice. Serum and kidneys were harvested. The concentrations of remdesivir and GS-441524 were measured using LC-MS/MS. Renal and liver function were assessed. Renal fibrosis was evaluated by Masson’s trichrome staining and Western blotting.

Results: Remdesivir and GS-441524 inhibited the expression of fibrotic markers (fibronecin and ASMA) in NRK-49F and HK2 cells. Intraperitoneal injection or renal injection of remdesivir attenuated renal fibrosis in UUO kidneys. Renal and liver function were preserved in remdesivir treated UUO mice. Two remdesivir metabolites were detected after injection. Phosphorylation of Smad3 that is enhanced in cell and animal models for renal fibrosis was attenuated by remdesivir. In addition, the expression of Smad7, an anti-fibrotic factor, was increased after remdesivir treatment in vitro and in vivo.

Conclusions: Remdesivir inhibits renal fibrosis in obstructed kidneys.

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PO2453

Heightened Innate Immune Response to COVID-19 Infection in CKD: Implications to Poorer Outcome During CKD
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Background: Meta-analyses reveal a significant association of chronic kidney disease (CKD) with severe COVID-19. The double stranded RNA virus SARS-CoV-2 can disease (CKD) with severe COVID-19. The double stranded RNA virus SARS-CoV-2 can cause kidney injury in individuals with pre-existing CKD and is a significant driver of end-stage renal disease in systemic lupus erythematosus patients. Complement activation, pro-inflammatory cytokine production, and the influx of macrophages have all been implicated in LN pathogenesis. The anaphylatoxin complement 5a (C5a) receptor 1 (C5ar1) is a major driver of the pro-inflammatory functions of complement activation. We examined C5ar1’s expression in kidney in lupus nephritides and investigated its role in controlling pro-fibrotic functions of macrophages.

Methods: C5ar1 expression, infiltrating immune cells, and fibrosis were examined by immunohistochemistry in LN patient kidney biopsies. M1 and M2 macrophages derived from human peripheral blood monocytes were used in in vitro assays to examine the effect of C5a stimulation and avacopan, a specific C5ar1 inhibitor, on the secretion of cytokines and other factors.

Results: In LN kidney biopsies, large numbers of macrophages, identified by CD68 staining, were observed in areas with severe fibrosis, and expressed C5ar1. In addition, C5ar1 was detected on distal tubules in biopsies of both normal and lupus nephritis kidneys. C5a increased the production of inflammatory cytokines TNFα and IL-6 from both M1 and M2 macrophages in vitro. Chemokines (MCP-3, MIP-1α, MIP-1b and MIP-3α), matrix metalloproteinases (MMP3 and MMP8), and pro-fibrotic growth factors (fibroblast activation protein, platelet-derived growth factor-A) were strongly increased in M2 macrophages with C5a stimulation, and these increases were blocked by the C5ar1 inhibitor avacopan.

Conclusions: C5ar1 activation induced macrophage secretion of factors that are known to cause inflammation, fibroblast activation and tissue fibrosis, and thus may contribute to LN disease progression. Inhibiting C5ar1 activity with avacopan blocks these pathological changes, and may provide therapeutic benefit to LN patients.

Funding: NIDDK Support

PO2454

Combined Soluble Epoxide Hydrolyse Inhibition and Epoxyeicosatrienonic Acid Administration Attenuates the Renal Fibrogenesis Without Additivity or Synergy
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Background: Epoxyeicosatrienonic acids (EETs) are arachidonic acid metabolites with biological effects, including anti-apoptotic, anti-inflammatory, and anti-fibrotic functions. Soluble epoxide hydrolase (sEH)-mediated hydrolysis of EETs to dihydroxyeicosatrienoic acids (DHETs) attenuates fibrotic functions. Recent studies have demonstrated inhibition of sEH prevents renal tubulointerstitial fibrosis and inflammation in chronic kidney disease (CKD) model. Here, we demonstrated the role and underlying mechanism of EETs in unilateral ureteral obstruction (UUO)-induced renal fibrogenesis.

Methods: Eight-week-old male wild type (Ephp2−/−) and Ephp2−/− mice underwent sham or UUO surgical procedures and were treated with the combination of 11,12- and 14,15-EETs (15 ug/kg/day, respectively) using osmotic pump for 7 days following UUO surgery.

Results: EETs administration abolished tubulointerstitial fibrogenesis, as demonstrated by reduced fibroblast activation and collagen deposition after UUO. Furthermore, inflammatory response was prevented as demonstrated by decreased macrophage infiltration and expression of inflammatory cytokines (TGF-β, IL-1β and IL-6) in EETs-administered UUO kidneys. The genetic inhibition of sEH also mitigated UUO-induced renal inflammation and interstitial fibrogenesis. The combination of EET administration and sEH inhibition also attenuated inflammation and renal interstitial fibrogenesis after UUO, but no additive or synergic effect of combined sEH inhibition and EETs administration.

Conclusions: Taken together, our findings provide that the underlying mechanism of EETs in kidney fibrogenesis during obstructive nephropathy, suggesting EETs as a potential therapeutic target of kidney fibrosis progression.

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PO2455

EP1 Receptor Antagonist Mitigates Early and Late-Stage Renal Fibrosis
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Background: Renal fibrosis is a hallmark of Chronic Kidney Disease (CKD), which affects 10-16% of the world’s adult population. Current treatment strategies are ineffective in attenuating renal fibrogenesis. Therefore, we are in urgent need for new therapeutic strategies against renal fibrosis. The cyclooxygenase/prostaglandin (COX/PG) system plays a key role in renal fibrosis and holds great promise as a suitable therapeutic target. Here, we used a translational approach to evaluate the role of the PGE1, EP1 receptor in the pathogenesis of renal fibrosis in several models of kidney injury, including human (fibrotic) kidney slices.

Methods: The anti-fibrotic effect of SC-19220 - an EP1 receptor antagonist - was studied in Madin-Darby Canine Kidney (MDCK) cells, mice subjected to seven days of unilateral ureteral obstruction (UUO), and healthy and fibrotic human precision-cut kidney slices (PKCS). Progression of fibrosis was evaluated on gene and protein level using qPCR, Western blot and immunohistochemistry.

Results: Pharmacological inhibition of the EP1 receptor using SC-19220 reduced TGF-β-induced fibrocyte (FN) expression, ERK1/2 phosphorylation and epithelial-to-mesenchymal transition in MDCK cells. Moreover, SC-19220 diminished fibrosis in UUO mice, measured by decreased protein expression of FN and α-smooth muscle actin (αSMA), and a reduction in collagen deposition. In addition, treatment of healthy human PKCS with SC-19220 reduced TGF-β-induced fibrocyte as shown by decreased gene levels of collagen 1A1, FN and oSMA as well as reduced collagen deposition. Moreover, similar observations were made using fibrotic human PKCS.

Conclusions: This study highlights that the EP1 receptor is a promising target for preventing both the onset and late stage of renal fibrosis. Moreover, we provide strong evidence that the effect of SC-19220 may translate to clinical care since its effects were observed in UOO mice and human kidney slices.

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