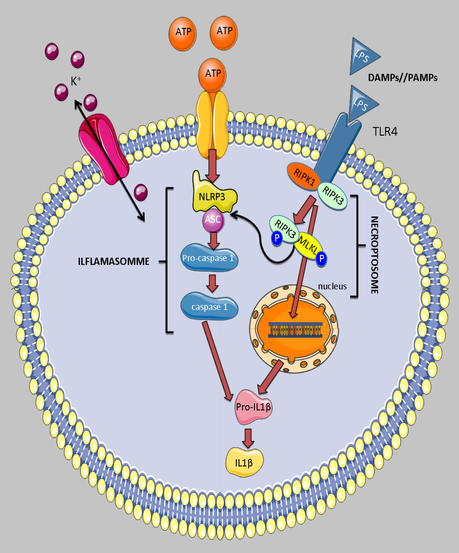
CCN2 GENE BLOCKADE IN VIVO AMELIORATES EXPERIMENTAL ACUTE RENAL INJURY.

Sandra Rayego-Mateos[1], Jose Luis Morgado-Pascual[1],Roel Goldschmeding[2]; Ana Belen Sanz[3]; Jesús Egido[3];Alberto Ortiz[3] and Marta Ruiz-Ortega[1].

[1] IIS-Fundación Jiménez Díaz. Universidad Autónoma de Madrid, Nephrology, Madrid, Spain;[2] University Medical Centre Utrecht, Pathology, Utrecht, Nederlands;[3] IIS-Fundación Jiménez Díaz, Nephrology, Madrid. Spain.

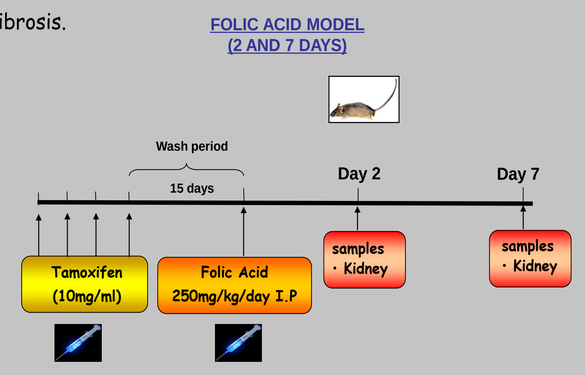
INTRODUCTION

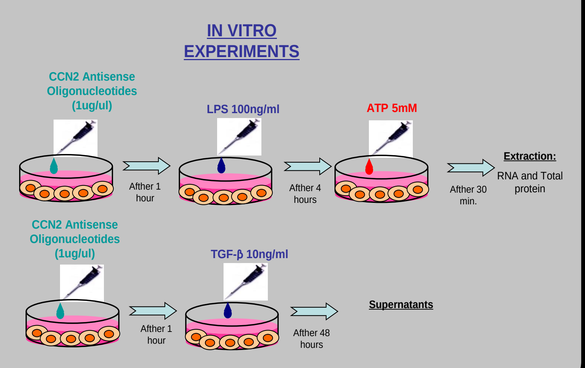
Acute kidney injury (AKI) and chronic kidney disease (CKD) are the most relevant forms of renal pathology. AKI is a multifactorial pathology characterized by renal tubular damage and inflammation. NLRP3 Inflamasome is a multiprotein complex of the immune system whose function is to recognize intracellular pathogens and induce the expression of pro-inflammatory cytokines as a response (IL-1β, IL-18 etc.). Previous studies on AKI have identified the death of tubular cells as a key factor in the development of this pathology and have located its origin in some type of necrosis associated with the inflammatory response such as necroptosis. There are several factors involved in the progression of CKD, including connective tissue factor (CTGF/CCN2), that are able to regulate inflammatory processes, angiogenesis as well as proliferation/apoptosis. In several experimental and human CKD, CCN2 has been considered as damage mediator and a potential therapeutic target, however there is no data about its role in AKI.



METHODS

The role of CCN2 in AKI was studied in a model of systemic administration of Folic Acid (FA) using a CCN2 deficient conditional mouse. Gene deletion of CCN2 was obtained by intraperitoneal injection of tamoxifen (10mg/ml; 4 injections) for 7 days followed by a two weeks washout period counting from the last day of injection. These animals were compared to the control group injected with the vehicle (corn oil). Mice were divided into two additional groups that received an intraperitoneal injection of Folic Acid (250 mg/kg/day) or vehicle, and renal damage was assessed 48 hours and 7 days later. In vitro studies were performed on murine tubuloepithelial cells stimulated with LPS for 4 hours + ATP for 30 min to evaluate inflammasome components, or murine fibroblastic cells NIH3T3 stimulated with TGF-β for 48hours to evaluate fibrosis.



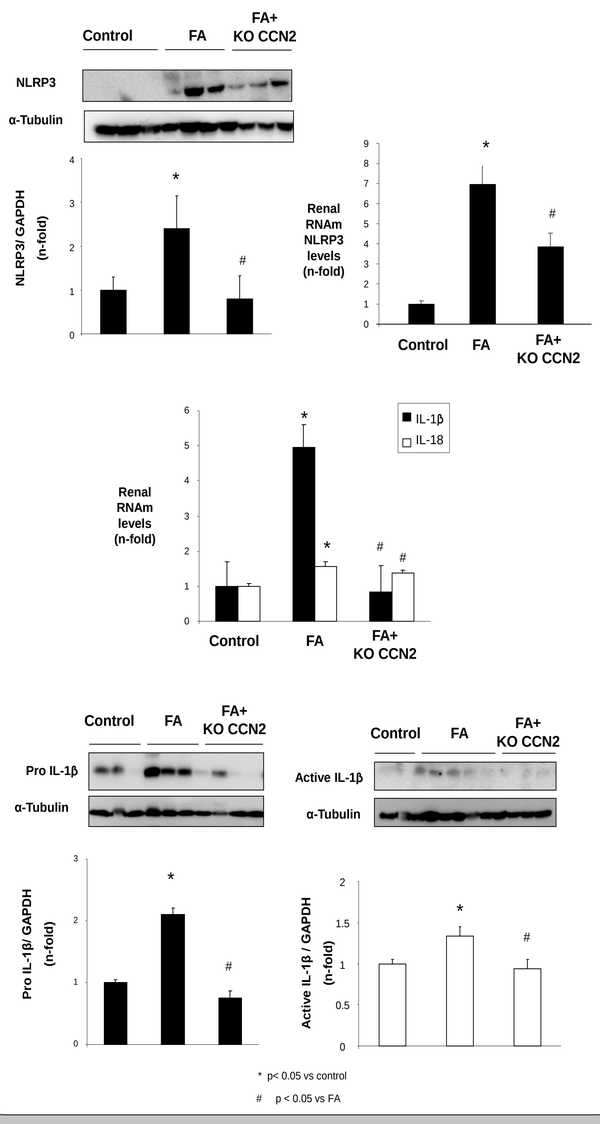


AIM

To study the role of CCN2 in the modulation of regulated necrosis/necroptosis as well as the NLRP3 inflammasome in a model of acute renal failure and in kidney cultured cells.

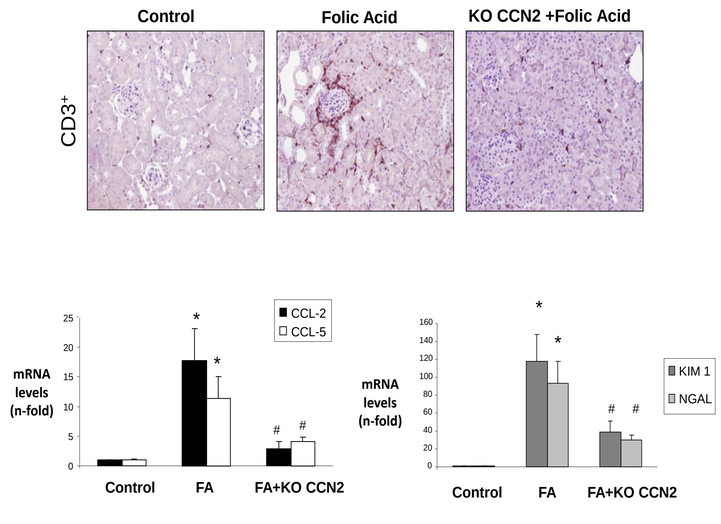
RESULTS

CCN2 gene blockade decreased NLRP3 inflammasome components at Gene/protein levels in the kidney of Folic Acid injected mice.

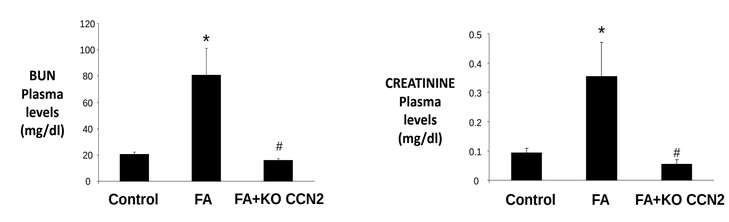


* p< 0.05 vs control
\# p < 0.05 vs FA

CCN2 gene blockade improved inflammation in AKI model induced by Folic Acid. Studies at 48 hours.

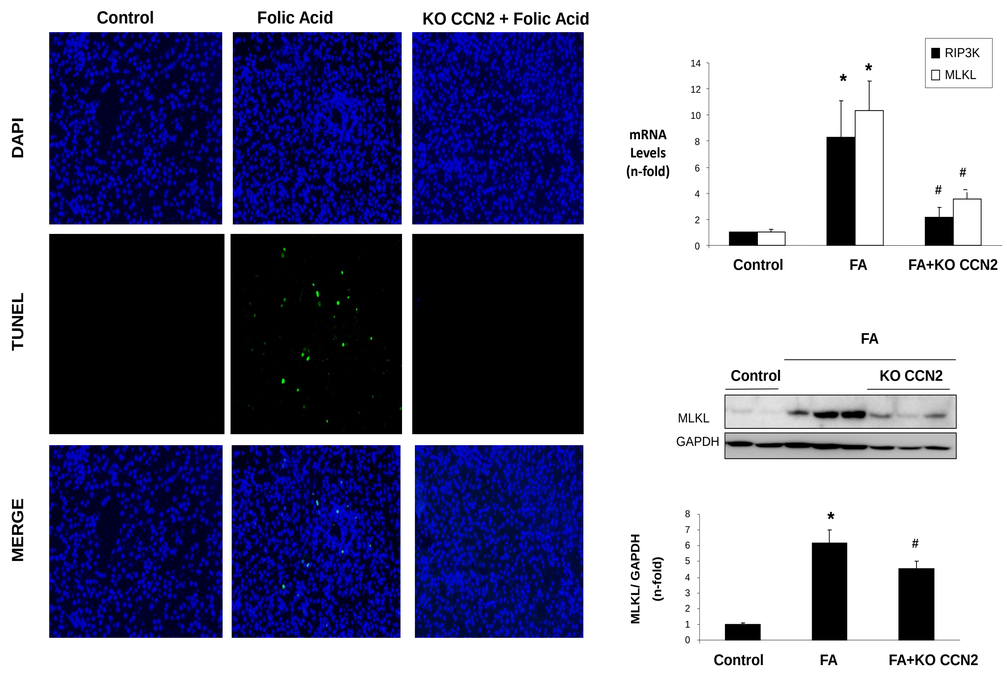


CCN2 gene blockade improved renal function in AKI model induced by Folic Acid. Studies at 48 hours.



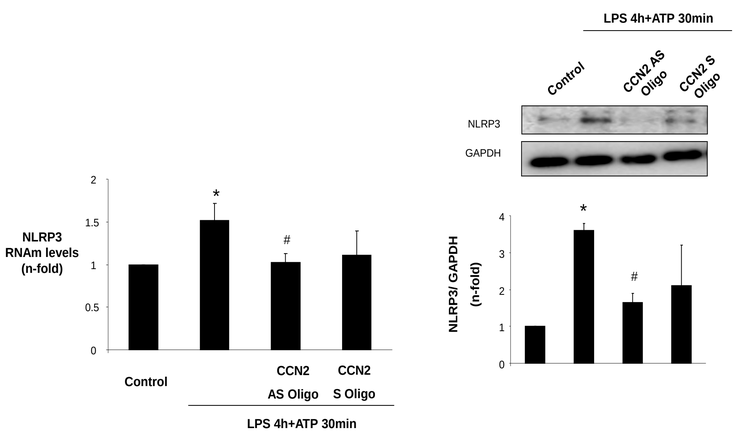
* p< 0.05 vs control
\# p < 0.05 vs FA

CCN2 Gene blockade decreased cell death/necroptosis in AKI model induced by Folic Acid. Studies at 48 hours.



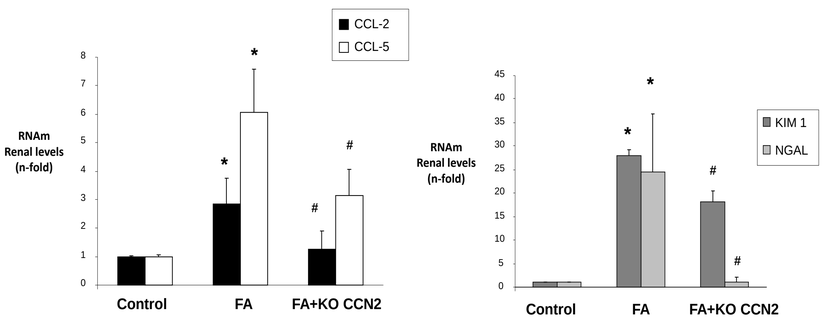
* p< 0.05 vs control
\# p < 0.05 vs FA

CCN2 Gene blockade decreased NLRP3 gene and protein expression in cultured MCT cells.



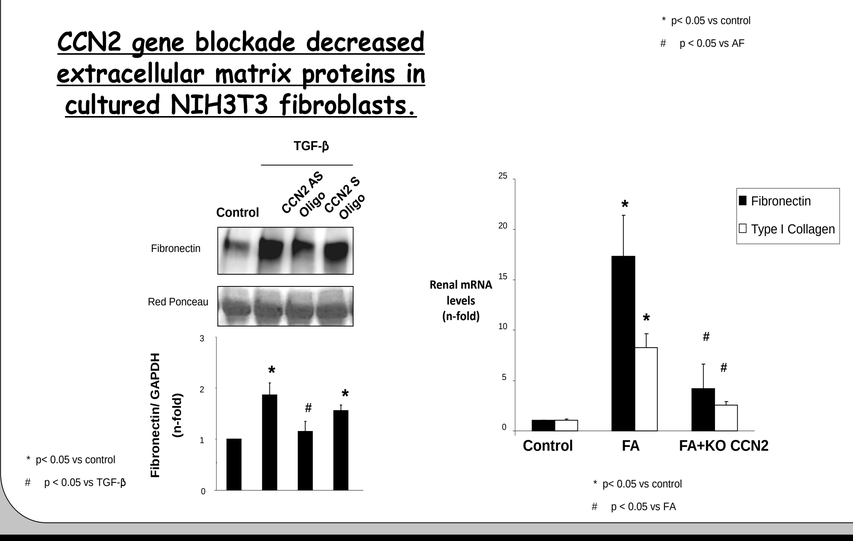
* p< 0.05 vs control
\# p < 0.05 vs LPS+ATP

CCN2 gene blockade decreased gene and protein expression of extracellular matrix proteins in a Folic Acid model. Studies at 7 days.



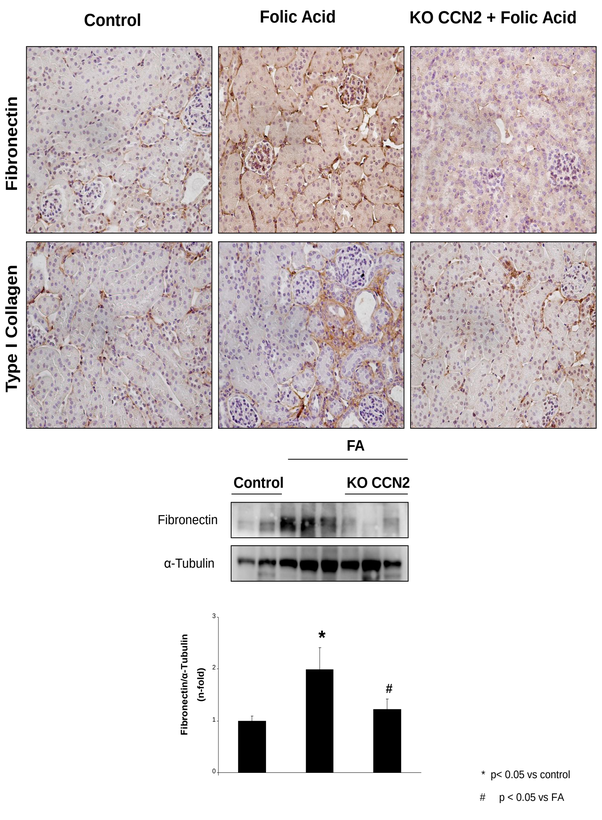
* p< 0.05 vs control
\# p < 0.05 vs AF

CCN2 gene blockade decreased extracellular matrix proteins in cultured NIH3T3 fibroblasts.



* p< 0.05 vs control
\# p < 0.05 vs TGF-β

* p< 0.05 vs control
\# p < 0.05 vs FA



* p< 0.05 vs control
\# p < 0.05 vs FA

CONCLUSION

CCN2 plays a key role in the activation of the NLRP3 inflammasome complex as well as in tubular cell death by necroptosis in folic acid-induced Acute Kidney Injury.

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Experimental AKI I
Sandra Rayego-Mateos
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