Upregulation of extracellular matrix protein expression by Cardiotrophin-1

Nuria Perretta-Tejedor[1,2,3], José M. Muñoz-Félix [1,2], Cristina Cuesta[1,2,3], Isabel Fuentes-Calvo[1,2], Nélida Eleno[1,3], José M. López-Novoa[1,2] , Carlos Martínez-Salgado[1,2,3]

[1]*Translational Research on Renal and Cardiovascular Diseases (TRECARD), Department of Physiology and Pharmacology, University of Salamanca,Spain;*
[2]*Institute of Biomedical Research of Salamanca (IBSAL), Spain;*
[3]*Institute of Health Sciences Studies of Castilla y León (IECSCYL), Research Unit, University Hospital of Salamanca, Spain*

INTRODUCTION

Renal tubule-interstitial fibrosis is characterized by myofibroblast proliferation and increased extracellular matrix (ECM) synthesis in the renal interstitium. TGF-β1 is one of the main profibrotic cytokines involved in this process. Cardiotrophin-1 (CT-1) is a member of the interleukin-6 family of cytokines. A protective effect of CT-1 was reported in several organs, promoting survival or antinflammatory effects. We have previously shown that CT-1$^{-/-}$ mice exhibit higher renal fibrosis than WT mice after 15 days of unilateral ureteral obstruction (UUO , an experimental model of tubulointerstitial-fibrosis. In addition, CT-1 treatment reduces renal fibrosis in WT mice.

AIM

The first aim of this study was to analyse the effect of CT-1 treatment in the severity of the renal tubule-interstitial damage induced by UUO in animals lacking CT-1 (KO). The second aim is to analyse the CT-1 and TGF-β1 induced effects on ECM protein synthesis in mouse embryonic fibroblast (MEFs) and in renal myofibroblasts obtained from obstructed kidneys in WT and CT-1 KO mice

MATERIALS AND METHODS

OUU was performed in CT-1$^{-/-}$ mice and their respective controls (WT) during 15 days – in order to evaluate renal tubule-interstitial fibrosis. We studied the effect of CT-1 treatment (100 μg/kg and 400 μg/kg) every 2 days. Interstitial fibrosis was evaluated by Masson´s trichrome staining. Myofibroblast marker (smooth muscle actin, α-SMA), and extracellular matrix proteins (collagen-1, fibronectin, CTGF) were evaluated by Western blot and qPCR. We also assessed collagen deposition in renal tissue by Sirius red staining. Moreover, cultured myofibroblasts were obtained from obstructed kidneys and stimulated with 40ng/ml and 100ng/ml CT-1 and with 1ng/ml TGF-β1 during 24 hours

RESULTS

Our data show higher expression of CT-1 in obstructed (O) than in non-obstructed (NO) kidneys after 15 days of UUO in WT mice (figure 1). CT-1 treatment (100 and 400 μg/kg) in CT-1 KO mice reduces collagen I, CTGF and fibronectin expression (figure 2), and tubulointerstitial fibrosis, assessed by Sirius red and Masson's trichrome staining (figure 3), as well as αSMA and PCNA expression (figure 4) in O kidneys after 15 days UUO.

On the other hand, our in vitro studies show that renal myofibroblasts express CT-1; stimulation with TGF-β1 increases this CT-1 expression (figure 5). Stimulation with CT-1 also increases expression of collagen I; however, co-stimulation with CT-1 and TGF-β1 blocked TGF-β1-induced expression of collagen I in MEFs (figure 6) and in renal myofibroblasts (figure 6). Moreover, in basal conditions and after TGF-β1 stimulation, CT-1 KO renal myofibroblasts show a higher expression of collagen I and fibronectin than WT cells (figure 7).

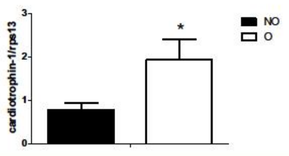

Fig 1. Analysis of CT-1 expression in O and NO from WT mice after 15 days of UUO evaluated by qPCR. *P<0,05

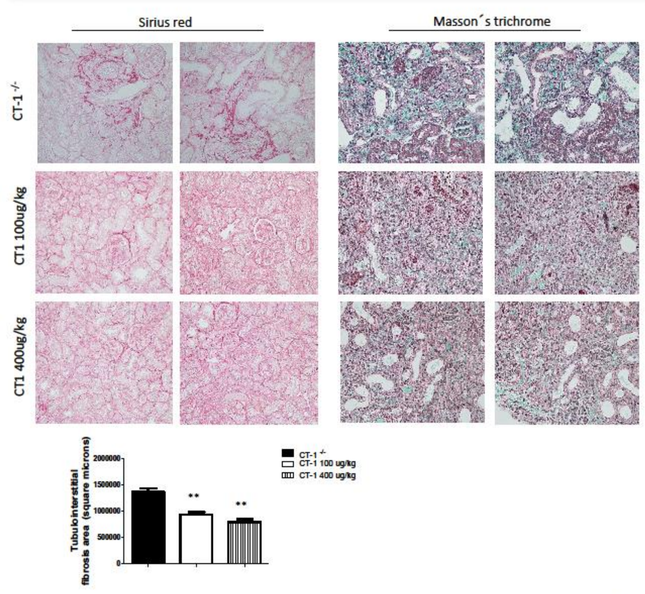

Fig 3. Analysis of tubulointerstitial fibrosis in O kidneys from CT-1$^{-/-}$ mice and after treatment with 100 mg/kg and 400 mg/kg CT-1 after 15 days of UUO, evaluated by Sirius red and Masson's trichrome staining. **P<0,01

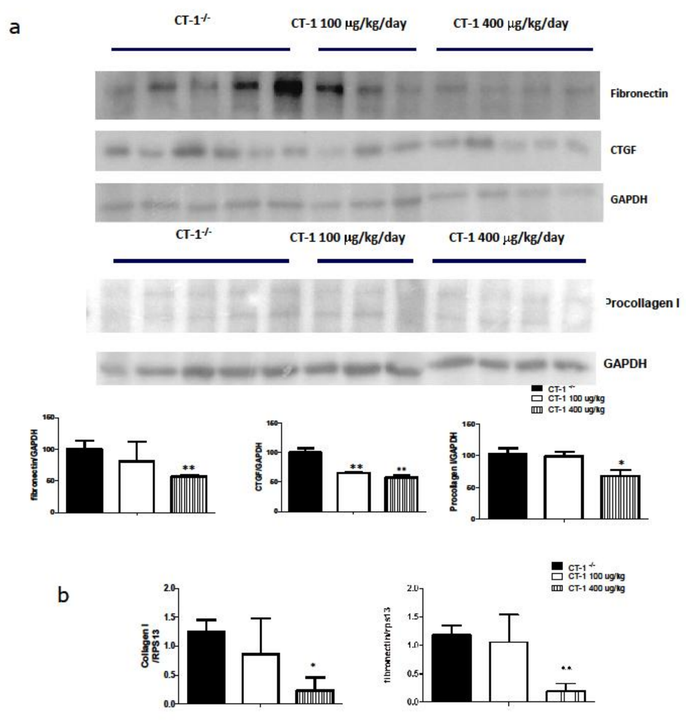

Fig 2. Analysis of collagen I, fibronectin and CTGF expression, in O kidneys from CT-1$^{-/-}$ mice treated with 100 μg/kg and 400 μg/kg CT-1 after 15 days of UUO, evaluated by western blot a) and by qPCR.b) *P<0,05 **P<0,01

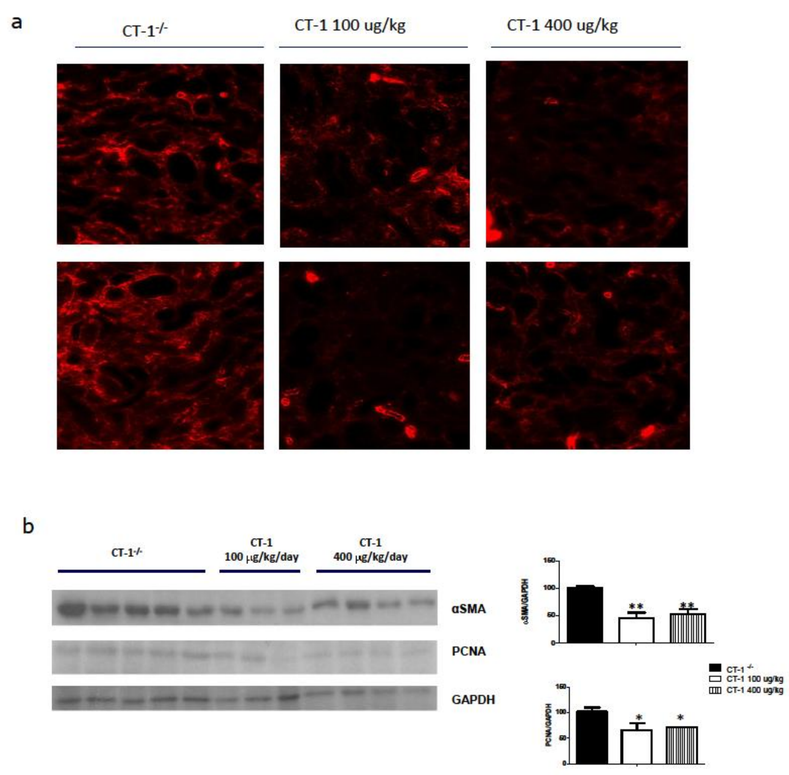

Fig 4. a) Inmunofluorescence of αSMA in O kidneys from CT-1$^{-/-}$ mice treated with 100 μg/kg and 400 μg/kg CT-1 after 15 days of UUO. B)Analysis of αSMA and PCNA expression in O kidneys from CT-1$^{-/-}$ mice treated with 100 μg/kg and 400 μg/kg CT-1 after 15 days of UUO, evaluated by western blot. *P<0,05 **P<0,01

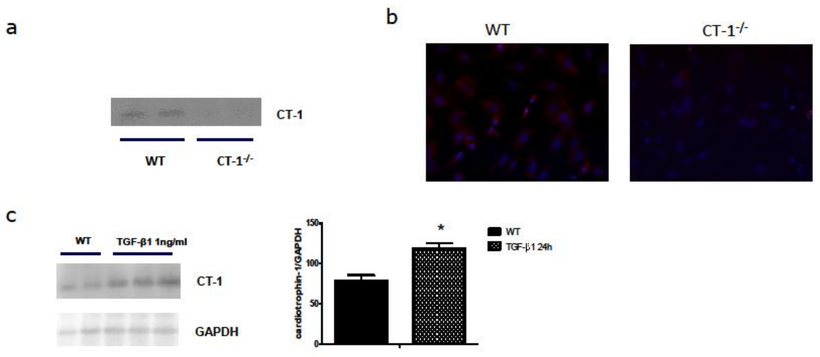

Fig 5.Analysis of CT-1 expression in renal myofibroblast from CT-1$^{-/-}$ and WT mice evaluated by western blot (a) and by immunofluorescence (b). Analysis of CT-1 expression in renal myofibroblasts from WT mice after TGF-β1 stimulation during 24 hours (c). *P<0,05

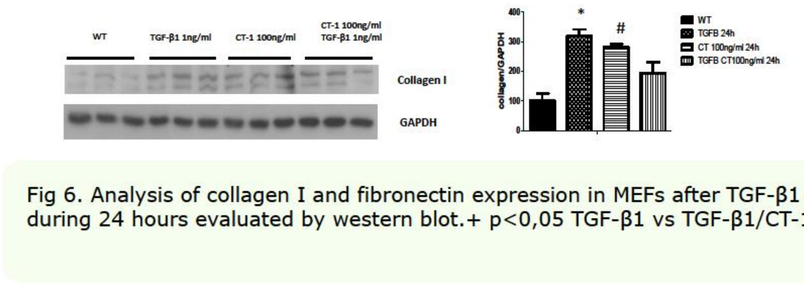

Fig 6. Analysis of collagen I and fibronectin expression in MEFs after TGF-β1 and CT-1 stimulation during 24 hours evaluated by western blot.+ p<0,05 TGF-β1 vs TGF-β1/CT-1 #p<0,05 WT vs CT-1

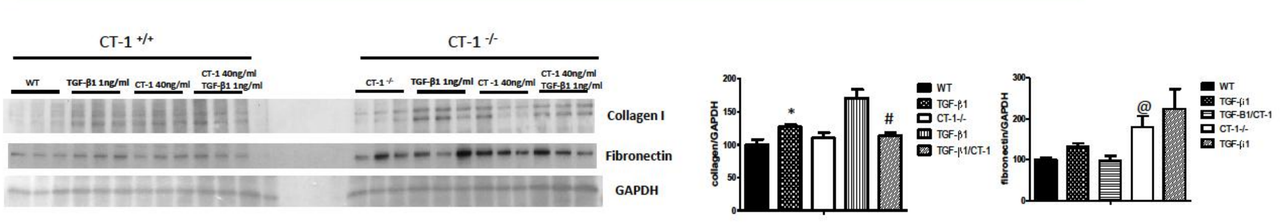

Fig 7.Analysis of collagen I and fibronectin expression in renal myofibroblasts from CT-1$^{-/-}$ and WT mice after TGF-β1 and CT-1 stimulation during 24 hours evaluated by western blot. +p<0,05 TGF-β1 WT vs TGF-β1 KO; # p<0,05 TGF-β1 vs TGF-β1/CT-1; @ P<0,05 WT vs KO

CONCLUSIONS

This study shows that CT-1 regulates renal fibrosis induced by obstructive nephropathy in KO mice and that endogenous CT-1 regulates the expression of matrix extracellular proteins in vitro. We suggest that the protective effects of CT-1 administration are related with a blockade of the pro-fibrotic TGF-β1 pathway.

53 ERA
99--MP
Renal pathology. Experimental and clinical.
Nélida Eleno
DOI: 10.3252/pso.eu.53era.2016
ePosters supported by F. Hoffmann- La Roche Ltd.
53rd CONGRESS ERA-EDTA VIENNA AUSTRIA MAY 21st-24th 2016
Held jointly with Österreichische Gesellschaft für Nephrologie
PosterSessionOnline.com