ndt

NEPHROLOGY DIALYSIS TRANSPLANTATION
Basic and clinical renal science

50th ERA-EDTA CONGRESS

MAY 18-21, 2013

Istanbul, Turkey Abstracts

www.era-edta2013.org

OXFORD OPEN

OXFORD
UNIVERSITY PRESS

www.ndt.oxfordjournals.org

Official Publication of the European
Renal Association -European Dialysis
and Transplant Association

RENAL FIBROSIS AND PROGRESSION

MO012 PBI-4050, A NOVEL FIRST-IN-CLASS ANTI-FIBROTIC COMPOUND, INHIBITS CTGF, α -SMA AND COLLAGEN EXPRESSION IN HUMAN FIBROBLASTS, AND REDUCES KIDNEY FIBROSIS IN 5/6-NEPHRECTOMIZED AND DOXORUBICIN-INDUCED NEPHROTOXICITY MODELS

Martin Leduc¹, Liette Gervais¹, Lilianne Geerts¹, Brigitte Groulx¹, François Sarra-Bournet¹, Alexandra Felton¹, Shaun Abbott¹, Jean-Simon Duceppe¹, Boulos Zacharie¹, Christopher Penney¹, Pierre Laurin¹ and Lyne Gagnon¹

¹ProMetric BioSciences Inc. Laval QC Canada

Introduction and Aims: Interstitial fibroblasts are the principal effector cells of organ fibrosis. Transforming growth factor (TGF)- β -activated myofibroblasts express α -smooth muscle actin (α -SMA), secrete connective tissue growth factor (CTGF) that functions as a downstream mediator of TGF- β action on fibroblastic cell types, and are important in the synthesis of collagen I. The aim of this study was to investigate the effect of PBI-4050, a first-in-class anti-fibrotic compound, on CTGF, α -SMA and collagen I expression in TGF- β 1-stimulated human fibroblasts (normal human dermal fibroblasts (NHDF)).

Methods: The effect of PBI-4050 on the mRNA expression of fibrotic markers was analyzed by qPCR in TGF- β 1-stimulated NHDF and in kidney from models of ESRD (5/6-nephrectomized rats) and AKI (doxorubicin-induced nephrotoxicity in mice).

Results: Activation of NHDF fibroblasts by TGF- β 1 resulted in a strong increase in mRNA expression of CTGF (30 times), α -SMA (10 times) and collagen I (2.5 times). PBI-4050 significantly reduced TGF- β 1-induced overexpression of CTGF (78%), α -SMA (85%) and collagen I (21%) mRNA in NHDF to the control level (untreated/no TGF- β stimulation). This is also translated at the protein level, as TGF- β 1-induced CTGF production is also significantly inhibited by PBI-4050 in NHDF. Furthermore, in non-stimulated NHDF, PBI-4050 reduced basal CTGF (60%), α -SMA (40%) and collagen I (25%) mRNA expression. These results correlate with *in vivo* inhibition of CTGF, α -SMA and collagen I mRNA expression observed in different models of kidney fibrosis. In 5/6-nephrectomized rats (CKD/ESRD model), oral administration of PBI-4050 (200 mg/kg) significantly decreased the mRNA overexpression of CTGF (50%), α -SMA (30%) and collagen I (50%) in the remnant kidney. In doxorubicin-induced nephrotoxicity (AKI model), oral administration of PBI-4050 (100 mg/kg) significantly decreased the overexpression of CTGF, α -SMA and collagen I mRNA expression close to the control level (no doxorubicin).

Conclusions: These results indicate a direct effect of PBI-4050 on fibroblasts as observed by an inhibition of CTGF, α -SMA and collagen I mRNA expression, and this is translated by a reduction of fibrosis in the kidney.

MO013 THE ROLE OF CALRETICULIN IN RENAL FIBROSIS

Niki Prakoura¹, Panagiotis Politis¹, Yoshito Ihara², Marek Michalak³ and Aristidis Charonis¹

¹Section of Histology Biomedical Research Foundation of the Academy of Athens Athens Greece, ²Wakayama Medical School Wakayama Japan, ³Department of Biochemistry University of Alberta Edmonton Canada

Introduction and Aims: Renal fibrosis is a key component of several kidney diseases, leading eventually to renal failure; however, the involved mechanisms are largely unknown. Using a classical proteomic approach on the Unilateral Ureteral Obstruction (UUO) rat model, we have found Calreticulin, a multifunctional Ca²⁺-binding protein, to be significantly upregulated both at the early and the late stages of fibrosis, especially in tubular epithelial cells. This work is focused on unraveling the role of Calreticulin in renal fibrosis, with studies both on cell-culture systems and animal models.

Methods: In order to further explore the role of calreticulin overexpression in the development of fibrosis, cell culture systems and animal models were used.

Results: Calreticulin overexpression in renal proximal tubular epithelial cells (HK-2) led to significant reduction of E-cadherin as well as increase of vimentin and vinculin

expression, without induction of α -SMA or collagen I, implicating a tendency for non-complete transformation of the epithelial cells. However, typical epithelial matrix proteins like fibronectin and collagen IV were significantly upregulated. Moreover, Calreticulin-overexpressing cells showed increased motility and cellular stress, reduced proliferation and increased sensitivity to apoptosis. These characteristics were also confirmed in the Calreticulin-overexpressing tubular cells in the UUO model. Mice heterozygous for Calreticulin were protected against the development of fibrosis in the UUO model. The appearance and size of the ligated kidneys were highly improved in the heterozygous mice compared with the wild-type ones. The Sirius Red staining showed significantly less collagen accumulation in the heterozygous mice, up to 17 days after the obstruction, in a time-dependent fashion. Also, both inflammatory/fibrotic markers and apoptosis were reduced in the heterozygous mice. Furthermore, the expression of thrombospondin 1, a key TGF- β -regulator, which is significantly induced in fibrosis, was ameliorated in the heterozygous mice, implicating an effect on TGF- β activation.

Conclusions: The findings described above strongly suggest that Calreticulin may be a novel important mediator of the fibrotic process and that its regulation of expression might be a future target of anti-fibrotic pharmacological interventions.

MO014 L-ENDOGLIN OVEREXPRESSION INCREASES RENAL FIBROSIS AFTER UNILATERAL URETERAL OBSTRUCTION

Bárbara Ujo^{1,2,3}, Jose M. Muñoz-Felix^{1,2,3}, Miguel Arevalo^{2,3,4}, Miguel Pericacho^{1,2,3}, María Gonzalez-Núñez^{1,2,3}, Carmen Langa⁵, Carlos Martínez-Salgado^{1,2,3}, Annette Düwell^{1,2,3}, Fernando Perez-Barriocanal^{1,2,3}, Carmelo Bernabeu⁵ and Jose Lopez-Novoa^{1,2,3}

¹Department of Physiology and Pharmacology University of Salamanca Salamanca Spain, ²Cardiovascular Research Biomedical Research Institute of Salamanca (IBSAL) Salamanca Spain, ³Institute Queen Sophie for Renal Research (IRSIN) Fundación Renal Iñigo Alvarez de Toledo Madrid Spain, ⁴Department of Anatomy and Histology University of Salamanca Salamanca Spain, ⁵Centro de Investigaciones Biomedicas (CIB) Consejo Superior de Investigaciones Científicas, (CSIC) Madrid Spain

Introduction and Aims: Transforming growth factor- (TGF-) plays a pivotal role in renal fibrosis. Endoglin, a 180 kDa membrane glycoprotein, is a TGF- β co-receptor which is overexpressed in several models of chronic kidney disease but its function in renal fibrosis remains still unclear. Two membrane isoforms generated by alternative splicing have been described, L-Endoglin (long, the most abundant isoform) and S-Endoglin (short) that differ from each other in their cytoplasmic tails. The aim of the present study was to assess the effect of L-Endoglin overexpression in renal tubulo-interstitial fibrosis.

Methods: For this purpose, a transgenic mouse which ubiquitously overexpresses human L-Endoglin (*L-ENG*⁺) was generated and unilateral ureteral obstruction (UUO) was performed in *L-ENG*⁺ mice and their wild type littermates. The degree of fibrosis was assessed by morphometric techniques and by the expression of fibrosis-related molecules (collagens, fibronectin) and the number of myofibroblasts. Renal fibroblasts were isolated and cultured from *L-ENG*⁺ and WT mice, and the fibrogenic potential of *L-ENG*⁺ fibroblasts was assessed by measuring collagen I and fibronectin production.

Results: Obstructed kidneys from *L-ENG*⁺ mice showed higher tubulo-interstitial fibrotic area and higher amounts of extracellular matrix proteins, such as type I collagen and fibronectin, than obstructed kidneys from WT mice. However, the higher increase of renal fibrosis observed in *L-ENG*⁺ mice is not due to a major abundance of myofibroblasts, as similar levels of α -smooth muscle actin were observed in obstructed kidneys from both mice types. Western blot analysis showed that levels of p-Smad1 and p-Smad3 were higher in O than in NO or sham kidneys, being these levels significantly higher in O kidneys from *L-ENG*⁺ than in those from WT animals. Accordingly, the number of p-Smad1-stained nuclei was higher in *L-ENG*⁺ than in WT mice. Western blot analysis revealed that collagen I and fibronectin production was higher in *L-ENG*⁺ fibroblasts than in wild type fibroblasts.

Conclusions: Our results suggest that L-Endoglin overexpression potentiates the activation of Smad1 and Smad3 pathways and this effect is associated to higher renal fibrosis development, probably based on a higher pro-fibrotic potential of *L-ENG*⁺ fibroblasts.