PEPSTATIN A IMPROVES RENAL FIBROSIS BY IMPAIRING UPA LYSOSOMAL RECYCLING

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Introduction and Aims: Chronic kidney disease (CKD) is characterized by interstitial fibrosis and tubular atrophy. With no current treatment and an increasing prevalence, new therapies are urgently needed. Renal fibrosis is caused by an excessive deposition of extracellular matrix proteins (ECM). Proteases play a crucial role contributing to this imbalance of the ECM homeostasis. Cathepsins B, L and K, which are lysosomal proteases, are known to play an important role on liver, lung and heart fibrosis respectively. However, the role of lysosomal cathepsins on kidney fibrosis remains unknown. Therefore, the aim of this study is to investigate the role of aspartyl lysosomal cathepsin D (Cts D) in renal fibrosis.

Methods: Cts D expression was determined in a panel of kidney biopsies by immunostaining. Unilateral Ureteral Obstruction (UUO) or chronic ischaemia reperfusion models of kidney fibrosis were performed for 15 days and 35 minutes/28 days respectively in 8-10 week C57BL/6 females. Animals were treated ± Cts D inhibitor (Pepstatin A) or Chloroquine (endo/lysosomal inhibitor) thrice weekly from day 4 post-surgery. Cts D activity was determined fluorimetrically. Fibrosis was assessed by Sirius Red (SR) staining and Collagen III and IV morphometry of renal cortex. No changes on collagen transcripts were detected, however, Pepstatin A increased collagen I degradation analysed by in situ zymography. Cts D blockade increased UPA expression and activity in both animal models determined by WB and plasminogen/casein zymography, providing a mechanistic link between Cts D and UPA on fibrotic tissue. Transcription of MMPs was increased by Pepstatin A treatment, providing a second mechanistic link between Cts D inhibition and the increase in collagen turnover.

Immunostaining and confocal microscopic for Cts D/UPA and UPA/LAMP-2 in fibrotic kidneys confirmed that Cts D and UPA co-localized inside the lysosomal compartment.

In order to further clarify the mechanism of reduced fibrosis in our models, HKC-8 cells were treated with Pepstatin A, Ca074-Me (cystein lysosomal cathepsin inhibitor) and chloroquine (endo/lysosomal inhibitor). Only Pepstatin A and chloroquine increased UPA secretion into the extracellular media, suggesting that inhibition of aspartyl lysosomal Cts D or lysosomal activity increased secreted UPA due to impaired recycling.

To confirm this hypothesis we tested the action of chloroquine or Pepstatin A in a 15 days UUO model. Both Pepstatin A and chloroquine successfully reduced renal fibrosis as assessed by SR morphometry on cortical sections.

Conclusions: Pepstatin A improves renal fibrosis in rodents by impairing UPA lysosomal recycling, which leads to an increase in ECM degradation.

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