renal ET pathway. To test this hypothesis, we measured urinary ET-1 excretion, inner medullary (IM) ET receptor (ETR) expression from mice on a NSD or 7 day high salt diet (7DHS) and the response of ET-1 mediated epithelial sodium channel (ENaC) activity in flox and CDNOS1KO mice. ET-1 excretion was similar between NSD flox and CDNOS1KO mice (0.14 ± 0.02 and 0.17 ± 0.06 pg/day, n = 10) and significantly increased similarly after a 7DHS (0.60 ± 0.1 and 0.60 ± 0.08 pg/day). IM ETR expression was similar between the mice on a NSD (~40% ETA, ~60% ETB receptors) and similarly shifted to ~95% ETB expression on a 7DHS. Basal CD ENaC open probability (Po) was similar (flox: 0.3 ± 0.08 CDNOS1KO: 0.3 ± 0.05). Acute ET-1 treatment significantly reduced ENaC Po from flox mice but not CDNOS1KO mice compared to basal (flox 0.1 ± 0.03 and CDNOS1KO 0.3 ± 0.05, n = 6 animals P < 0.05). In conclusion, CD NOS1 appears not to regulate renal ET-1 production or ETR expression. However, the mechanism of ET-1 inhibition of CD ENaC is via NOS1. We propose that the salt-dependent increase in BP and Na retention observed in CDNOS1KO mice is mediated by the loss of ET signaling in the CD.


Increased of heparanase expression in hypoxic endothelial cells and kidney ischemic-reperfusion injury associates with endothelin-1 elevation and eNOS reduction

Nur Arfian¹, Keiko Yagi², Kazuhiko Nakayama³, Dwi C. Ratna Sari, Muhammad M. Romi, Untung Tranggono, Harry S. Muliawan, Noriaki Emoto

¹Anatomy, Embryology, and Anthropology Department, Faculty of Medicine, Gadjah Mada University, Jogjakarta, Indonesia
²Clinical Pharmacy Department, Kobe Pharmaceutical University, Kobe, Japan
³Cardiovascular Medicine Division, Internal Medicine Department, Graduate School of Medicine, Kobe University, Kobe, Japan
⁴Surgery Department, Faculty of Medicine, Gadjah Mada University, Jogjakarta, Indonesia
E-mail address: n_arfian@yahoo.com (N. Arfian)

Renal ischemia/reperfusion injury (I/R) is the most frequent cause of acute kidney injury. It has been reported that endothelin-1 deletion from endothelial cells attenuates I/R injury. Heparanase is an enzyme that degrades endothelial surface layer and induces endothelial injury. The association between heparanase and ET-1 in kidney I/R is still unclear. We induced hypoxic condition for 30 min in a MS-1 endothelial cell culture using a hypoxic bag. We extracted RNA and quantified pre-pro-ET1, heparanase, endothelial nitrite oxide synthase (eNOS) and ICAM-1. To examine heparanase contribution in I/R, we performed a kidney I/R injury model in black-six mice (n = 7) using renal pedicle clamping for 30 min and sacrificed the mice in 1, 3 and 24 h after operation. Sham-operation procedure (SO, n = 5) was used as control. PAS was used to quantify tubular injury score. Serum creatinine was quantified from orbital venous. We did immunostaining for heparanase and double glycocalyx-von Willebrand factor to elucidate the contribution of heparanase in the early step of ischemic acute kidney injury. Western-blotted was used to analyze eNOS expression. Ischemia induced a significant increase of pre-proET1 and heparanase mRNA expression that was associated with ICAM-1 elevation and eNOS reduction. In-vivo, we found elevation of heparanase mRNA expression in the early stage of I/R injury (1, 3 and 24 h). This is associated with increase of tubular injury score, creatinine serum level and eNOS reduction. In a further analysis, EC derived ET-1 significantly reduced heparanase mRNA (p < 0.05) expression after kidney I/R injury. In this study, we suggested that heparanase might contribute to the ET-1 effect in inducing endothelial injury in hypoxic and kidney I/R condition.


Endothelial cell-derived endothelin-1 exaggerates kidney fibrosis through ETAR activation in renal interstitial cells

Nur Arfian¹, Keiko Yagi², Kazuhiko Nakayama³, Nicolas Vignon-Zellweger⁴, Susi Heiden⁵, Tran V. Hung⁶, Harry S. Muliawan, Gahan Satwiko, Noriaki Emoto

¹Anatomy, Embryology, and Anthropology Department, Faculty of Medicine, Gadjah Mada University, Jogjakarta, Indonesia
²Clinical Pharmacy Department, Kobe Pharmaceutical University, Kobe, Japan
³Cardiovascular Medicine Division, Internal Medicine Department, Graduate School of Medicine, Kobe University, Kobe, Japan
E-mail address: n_arfian@yahoo.com (N. Arfian)

Kidney fibrosis is a final pathway of chronic kidney disease (CKD) and characterized by myofibroblast formation from renal interstitial cells. The clear mechanism of how endothelin-1 and its receptors involved in CKD cause interstitial cell proliferation is still unknown. We performed unilateral ureteral obstruction (UUO) in vascular endothelial endothelin-1 knock-out (VEETKO, n = 7) and WT mice (n = 7), which were then sacrificed in days 3 and 14. We observed renal fibrosis, the myofibroblast area, and the capillary number using Sirius Red, α-SMA, and CD31 immunostaining. Double α-SMA and PDGFRβ staining and quantification were done to examine interstitial cell expansion. Renal blood flow was observed and quantified by laser Doppler imaging. Western blot was done to examine α-SMA, PDGFRβ and TGFβ1 expression. Kidney ET-1 system was measured using ELISA and real time PCR. Double α-SMA and ETAR immunostaining was done to elucidate ETAR in myofibroblast cells. We found significantly lower fibrosis, myofibroblast area, and TGFβ1 expression (p < 0.05) in VEETKO mice compared to WT mice. Kidney ET1 and pre-pro ET1 mRNA levels increased after UUO, however were significantly lower in VEETKO mice. VEETKO mice also had significantly lower interstitial cell expansion and myofibroblast area compared to WT mice. EC derived ET-1 deletion also improved renal blood flow and capillary number (p < 0.05) after UUO. We observed ETAR expression in the myofibroblast area and is colocalized with PDGFRβ. EC derived ET-1 deletion attenuates kidney fibrosis via preserving the capillary and reducing interstitial cell expansion and myofibroblast formation. ETAR from interstitial cells may induce proliferation and myofibroblast formation. Targeting the ET-1 and ETAR axes in EC and interstitial cell may give the best approach to treat kidney fibrosis.


Hypoxia stimulates glomerular reactive oxygen species through an endothelin-1/ET-A dependent mechanism

J. Brett Heimlich, Paul M. O’Connor, Dao H. Ho, Steffen E. Meiler, David M. Pollock

²Section of Experimental Medicine, Department of Medicine, Georgia Regents University, Augusta, GA, USA
³Department of Anesthesiology and Perioperative Medicine, Georgia Regents University, Augusta, GA, USA
E-mail address: jheimlich@gru.edu (J.B. Heimlich)