Abstract: P702

Anti-thrombin nanoparticles for reduce vascular damage and promote functional recovery in acute ischemic kidney injury well after reperfusion

Authors:
H Pan¹, R Grabau¹, I Vargas¹, M Baldwin¹, B Cara¹, D Stephenson¹, A Lindon¹, C Chalfant¹, S Wickline¹,
¹University of South Florida, The Heart Institute - Tampa - United States of America,

Topic(s):
Anticoagulants

Citation:
Funding Acknowledgements:
DK102691

Introduction: We have shown previously that pretreatment of acute ischemic kidney injury (AKI) in mice prior to reperfusion with anti-thrombin perfluorocarbon nanoparticles (PFC NP) limits damage to endothelium and hastens functional recovery. However, whether such treatments are effective after AKI is established is not known. We hypothesized that thrombin would continue to exert deleterious clotting and molecular signaling effects in AKI well after reperfusion that would respond to sustained local inhibition with long acting anti-thrombin nanoparticles.

Methods: 23 C57Bl6 mice underwent bilateral kidney ischemia for 17 min, followed by 2 hours reperfusion and i.v. injection of anti-thrombin PPACK (D-phenylalanyl-L-prolyl-L-arginine chloromethyl ketone)-conjugated PFC NP (~ 13,000 PPACK per PFC NP), or plain PFC NP (control: no drug). At 24 hours BUN was measured, and mice were euthanized for kidney histological assessment (H&E), protein expression (western blot) and eicosanoid mediators of inflammation (LC-MS/MS: AB SCIEX 5500 QTRAP).

Results: BUN at 24 hours after AKI was 63.29±9.09 vs 110.96±6.21 (P < 0.002), for treated versus untreated mice, respectively, a 43% improvement. Western blots (Figure) indicated 40% reduction of canonical NF-kB signaling pathway protein p65 (p < 0.01) and 2.2 fold increases in Bcl-xL: Bax ratio (P < 0.01). Vascular damage, as indicated by glomerular and mesangial hemorrhage (Figure), was reduced, as was tubular cell swelling and edema. Levels of inflammatory procoagulant eicosanoids (e.g., PGE1, TBX2, PGA2, 15-HETE, 5-HETE, etc.) generally were higher in renal medulla than in cortex, and were suppressed by PPACK PFC NP.

Discussion: Continued inhibition of thrombin in AKI with locally-acting PPACK PFC NP preserved vascular integrity, limited renal hemorrhage, mitigated inflammation and tubular cell death, and accelerated functional recovery even when administered 2 hours after reperfusion. Because these PPACK PFC NP do not prolong bleeding times or coagulation parameters beyond ~30-60 min after injection, yet maintain prolonged local surveillance against activated thrombin, they represent a potentially useful therapeutic strategy for established AKI after an ischemic insult.
Abstract:
Anti-thrombin nanoparticles for reduce vascular damage and promote functional recovery in acute ischemic kidney injury well after reperfusion

Authors:
H Pan 1, R Grabau 1, I Vargas 1, M Baldwin 1, B Cara 1, D Stephenson 1, A Lindon 1, C Chalfant 1, S Wickline 1

1 University of South Florida, The Heart Institute - Tampa - United States of America,

Introduction: We have shown previously that pretreatment of acute ischemic kidney injury (AKI) in mice prior to reperfusion with anti-thrombin perfluorocarbon nanoparticles (PFC NP) limits damage to endothelium and hastens functional recovery. However, whether such treatments are effective after AKI is established is not known. We hypothesized that thrombin would continue to exert deleterious clotting and molecular signaling effects in AKI well after reperfusion that would respond to sustained local inhibition with long acting anti-thrombin nanoparticles.

Methods: 23 C57Bl6 mice underwent bilateral kidney ischemia for 17 min, followed by 2 hours reperfusion and i.v. injection of anti-thrombin PPACK (D-phenylalanyl-L-prolyl-L-arginine chloromethyl ketone)-conjugated PFC NP (~13,000 PPACK per PFC NP), or plain PFC NP (control: no drug). At 24 hours BUN was measured, and mice were euthanized for kidney histological assessment (H&E), protein expression (western blot) and eicosanoid mediators of inflammation (LC-MS/MS: AB SCIEX 5500 QTRAP).

Results: BUN at 24 hours after AKI was 63.29±9.09 vs 110.96±6.21 (P < 0.002), for treated versus untreated mice, respectively, a 43% improvement. Western blots (Figure) indicated 40% reduction of canonical NF-kB signaling pathway protein p65 (p < 0.01) and 2.2 fold increases in Bcl-xL: Bax ratio (P < 0.01). Vascular damage, as indicated by glomerular and mesangial hemorrhage (Figure), was reduced, as was tubular cell swelling and edema. Levels of inflammatory procoagulant eicosanoids (e.g., PGE1, TBX2, PGA2, 15-HETE, 5-HETE, etc.) generally were higher in renal medulla than in cortex, and were suppressed by PPACK PFC NP.

Discussion: Continued inhibition of thrombin in AKI with locally-acting PPACK PFC NP preserved vascular integrity, limited renal hemorrhage, mitigated inflammation and tubular cell death, and accelerated functional recovery even when administered 2 hours after reperfusion. Because these PPACK PFC NP do not prolong bleeding times or coagulation parameters beyond ~30-60 min after injection, yet maintain prolonged local surveillance against activated thrombin, they represent a potentially useful therapeutic strategy for established AKI after an ischemic insult.