Recombinant Alkaline Phosphatase modulates inflammation and injury in two rat models of AKI

Bülent Ergin1, Asli Kandili2, Ebru Gurel2, Andrea van Elsas3 and Can Ince1. 1) Department of Translational Physiology, Academic Medical Center, University of Amsterdam, Netherlands; 2) Department of Biology, University Istanbul, Turkey; and 3) AM Pharma, Bunnik, Netherlands.


Abbreviations used

AKI – acute kidney injury  
BAX – Bcl-2-associated X protein  
IL-6 – interleukin 6  
INOS – inducible NO synthase  
I/R – ischemia/reperfusion  
L-FABP – L-fatty acid binding protein  
LPS – lipopolysaccharide  
MAP – mean arterial pressure  
MO – monocytic origin  
PK – protein kinase  
PKC – protein kinase C  
Pp – post prandial  
RVR – renal vascular resistance  
RVR – renal vascular resistance

In rats, mild (I/R) or severe (LPS) acute kidney injury was induced and kidney disease followed for up to 3 h post-treatment. A single i.v. dose of 1000 U/kg recAP modulated markers of:  
- Renal vascular response (RVR)  
- Leukocyte infiltration and inflammation (MPO, INOS, IL-6)  
- Renal injury and apoptosis (L-FABP, NGAL, Bax)

In the context of disease severity induced, and within the 3 h time-frame of the experiment, oxygenation (cortical, medullar O2 delivery and consumption) and tubular function (Creatinine Clearance, FENa) were not modulated. Therefore it was concluded that in these animals RecAP inhibited acute renal inflammation and markers of tissue damage.

Fig 1. A novel recombinant human intestinal alkaline phosphatase, recAP, consists of the stable crown domain of human Placental AP (red), fused to the activity domain of human Intestinal AP. (Kifer-Morina et al., 2014)

Compared to BAP, recAP retains all enzyme activity but gains in stability and PK properties. To study the activity and mode-of-action of recAP, the protein was applied to rats during the first hours following induction of acute kidney by ischemia-reperfusion (I/R model) or LPS injection (Septic shock model; Legrand et al., 2011). Rats were kept warm and received fluid resuscitation as required for the duration of the experiments. Animals were instrumented for real-time assessment of hemodynamics. Kidneys were exposed to quantify oxygen delivery and expenditure. Blood and urine was collected for biochemical analysis and inflammatory kidney damage markers.

Fig 2. Schematic set-up of the I/R induced rat AKI experiment. Within the first few hours post-induction real-time data on hemodynamics and oxygen consumption were collected and tissue harvested for analysis of markers of inflammation and kidney damage.

Fig 3. Hemodynamic response to I/R and treatment with recAP. Semi-therapeutic administration of recAP demonstrated little effect on systemic hemodynamics (MAP), but significantly affected renal vascular resistance immediately post-reperfusion.

Fig 4. Kidneys harvested 3 hours post-treatment were stained for various markers of inflammation (INOS, IL-6) and renal injury response (L-FABP, Bax). Staining was quantified using histological scoring methodology (HSICORE). RecAP inhibited INOS and IL-6. Damage markers were not induced by I/R and not regulated by recAP.

Fig 5. Schematic set-up of the LPS-induced rat AKI experiment. Within the first few hours post-induction real-time data on hemodynamics and oxygen consumption were collected and tissue harvested for analysis of markers of inflammation and kidney damage.

Fig 6. Hemodynamic response to LPS and treatment with recAP. Semi-therapeutic administration of recAP demonstrated little effect on systemic hemodynamics (MAP), but significantly affected renal blood flow.

Fig 7. Kidneys harvested 3 hours post-treatment were stained for various markers of inflammation (MPO, IL-6) and renal injury response (L-FABP, Bax). Similar data were recorded for INOS and NGAL. RecAP completely inhibited LPS mediated induction of neutrophil infiltration (MPO), inflammation (INOS, IL-6), and injury (L-FABP, NGAL, Bax).

References