Alkaline phosphatase and acute kidney injury

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Acute kidney injury (AKI)

- AKI incidence in the Western World is 2 million patients / year\(^1\)
- Major cause of AKI is sepsis\(^2\)

\(\text{Drug & Contrast Toxins}
\)
AKI incidence 1-7%
AKI mortality 20-50%

\(\text{Sepsis}
\)
AKI incidence 20-60%
AKI mortality 30-70%

\(\text{Cardiovascular Surgery}
\)
AKI incidence 3-12%
AKI mortality 20-45%

\(\text{Other}
\)
A/o hemorrhage, trauma

- Current treatment: renal replacement therapy
- No drugs licensed to treat AKI

Alkaline phosphatase (AP)

- Dephosphorylating enzyme
- Detoxifying properties
- Two phase II clinical trials showed improved renal function with AP in patients with sepsis-induced AKI

References:
Alkaline phosphatase treatment improves renal function in severe sepsis or septic shock patients

Suzanne Heemskerk, PhD; Rosalinde Masereeuw, PhD; Olof Moesker; Martijn P. W. J. M. Bouw; Johannes G. van der Hoeven, MD, PhD; Wilbert H. M. Peters, PhD; Frans G. M. Russel, PhD; Peter Pickkers, MD, PhD; on behalf of the APSEP Study Group

Alkaline phosphatase for treatment of sepsis-induced acute kidney injury: a prospective randomized double-blind placebo-controlled trial

Mechanism of action
Lipopolysaccharide (LPS)
Toll-like receptor 4 (TLR4)

LPS

Mechanism of action
Adenosine

Mechanism of action
Adenosine

Aim

To investigate the role of alkaline phosphatase and adenosine receptor A2B in endotoxemia-induced acute kidney injury
Method (TLR4 pathway)

*In vitro* experiments - ciPTEC

Human proximal tubular epithelial cell model (ciPTEC) ¹

- Stimulate cells with AP and LPS to study inflammatory response (TNF-α, IL-1β, IL-6, IL-10)
- Investigate expression and localization of TLR4

Characteristics conditionally immortalized conditionally immortalized proximal tubular epithelial cell line

Morphology

Phase contrast microscopy: Primary cells (heterogenous) Before immortalization and subcloning

Phase contrast microscopy: Confluent monolayers after immortalization, subcloning and maturation at 37 degrees

Electron micrograph: microvilli
Western blotting: expression of aquaporin-1, dipeptidyl peptidase V and multi resistant protein 4

Sodium-dependent phosphate uptake in ciPTEC: Black line = presence of sodium, Dashed line = with N-methyl-D-glucamine as sodium replacement
Method (TLR4 pathway)

*In vitro* experiments – ciPTEC (n=3)

$t = -2$
$t = 0$
$t = 6$

*Beumer et al. (not published)*

*Heemskerk et al, J Biomed Biotechnol (2010)*
Results (TLR4 pathway)

*In vitro* experiments – ciPTEC (n=3)

- IL-10 and IL-1β not detectable
- TLR4 was expressed

* p<0.05 compared to control
# p<0.05 compared to dLPS
Method (adenosine)

In vivo experiment – A2BR⁻/⁻ mice
Performed in Denver, Colorado, USA

- Male and Female C57BL/6 mice
  - Aged 15 – 21 weeks
  - Weighed 20 – 26 gram
- Each group: 50% Female, 50% Male
- Histology of Kidney (periodic acid-Schiff-staining: PAS)
- Serum creatinine and urea (quantitative colorimetric assays)
- Sacrificed after 24 hours: heart puncture
- Serum and renal cytokines Collected (Luminex and ELISA)
- Kidney: mRNA expression of adenosine receptors (qPCR)

Adenosine receptor A2B deficient Mice (n=10)

Plac. (n=4)

LPS (n=6)

Wild Type (WT) Mice (n=4)

Plac. (n=2)

LPS (n=2)
Results (adenosine)

*In vivo* experiment – A2BR<sup>−/−</sup> mice

Serum

![Graphs showing cytokine levels in different groups](image-url)
Results (adenosine)

*In vivo* experiment – A2BR\(^{-/-}\) mice

Kidney
Conclusion

- **Alkaline Phosphatase can remove phosphate from LPS**
  - Exerts anti-inflammatory properties: systemically and in the kidney
  - LPS induced TNF-α production is reduced by AP
  - Suitable model to study LPS-TLR4 pathway

- **Alkaline Phosphatase can remove phosphate from ATP**
  - Potent protective role of adenosine receptor A2B during systemic inflammation
Questions