



Alkaline Phosphatase for treatment of Acute Kidney Injury

INTRODUCTION

AM-Pharma has shown in a Phase-II trial that treatment with Alkaline Phosphatase can improve survival of patients with Acute Kidney Injury. Mortality rate in patients with renal failure secondary to sepsis and treated with AP was reduced from 60% (placebo group) to 27% (AP group). AM-Pharma is aiming for AP to be the first approved drug in Acute Kidney Injury.

Hospital-acquired Acute Kidney Injury (AKI) occurs in as many as 4% of hospital admissions and 25% of critical care admissions. The incidence is increasing because of an aging population, the increasing exposure to nephrotoxic drugs in hospitals, increased hospital infections and increasing number of surgical interventions. Acute Kidney Injury (AKI) as a result of sepsis occurs in nearly half of the 2 million sepsis patients in the ICU setting and is associated with a high rate of mortality (20-50%). According to the US Center for Disease Control, the incidence of sepsis has risen 329% over the past 20 years and is expected to increase further.

Since there is no drug on the market to treat AKI, we believe that AP may provide significant benefit to the 2 million patients that experience AKI annually. These benefits are:

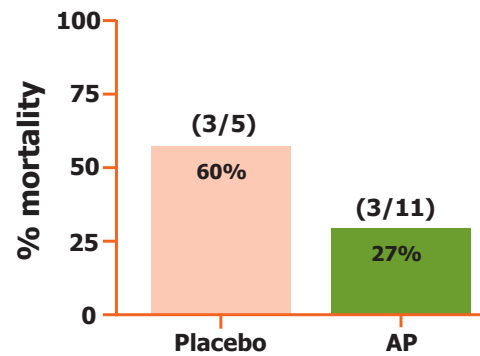
- decreased mortality rate
- decreased need for dialysis
- reduction of the dialysis-associated morbidity

CLINICAL RESULTS

Efficacy in acute kidney injury in a sepsis patient population

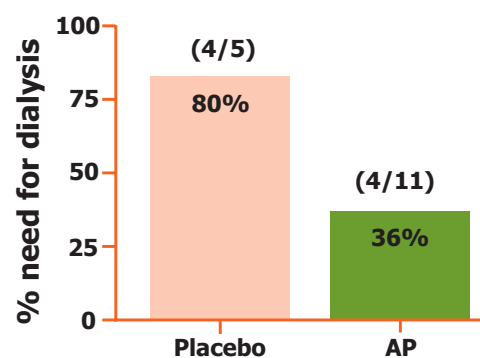
AM-Pharma has conducted a phase-II study with bovine intestinal AP in 36 patients with sepsis and organ failure admitted to Intensive Care Units. The largest organ failure group was "sepsis with acute kidney injury (AKI)" (45% of patients). AKI was defined as serum creatinine > 150µmol/L. The treatment consisted of a 24 hrs infusion of AP. The treatment was well tolerated. No drug-related SAEs were reported in the AP group.

Figure 1. The percentage mortality in sepsis patients with AKI that received either placebo or AP.



The all-cause mortality in patients with AKI (controls) was 60% whereas in AP-treated patients all-cause mortality was 27% (figure 1). However, although all-cause mortality was improved, survival in AKI is dependent on the severity of renal damage for which dialysis (based on creatinine progression, indicator of severity and risk) is the key treatment. Dialysis requirement was reduced from 80% (controls) to 36% (AP-treated patients) in the study (figure 2). The results were supported by serum creatinine data and renal markers.

Figure 2. The percentage of patients with Acute Kidney Injury that required dialysis. AP versus placebo.



AP effect on renal function parameters

In this study several renal parameters were evaluated that have contributed to the understanding of the AP effects in this patient population. AP treated patients demonstrated an attenuated increase in cumulative urinary NO metabolite excretion, whereas excretion of NO metabolites increased in placebo treated patients. Baseline expression levels of iNOS in renal cells were



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42-fold induced (vs. healthy subjects), and AP reduced this induction with 80±5%. Plasma creatinine clearance improved in patients treated with AP and deteriorated in placebo treated patients.

During the first 24 hrs, the amount of glutathione-S-transferase-A1, a marker for proximal tubular damage, in urine of AP treated patients decreased with 70% compared to an increase with 200% in placebo treated patients. This correlated with cumulative urinary NO metabolite excretion, indicating NO-induced proximal tubular damage.

Overall we conclude that the AP has a protective effect on the damage of the proximal tubules of the kidney, and that treatment of sepsis patients with AKI correlates positively with the survival of these patients.

Results of this trial were recently published in Critical Care Medical Journal:

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

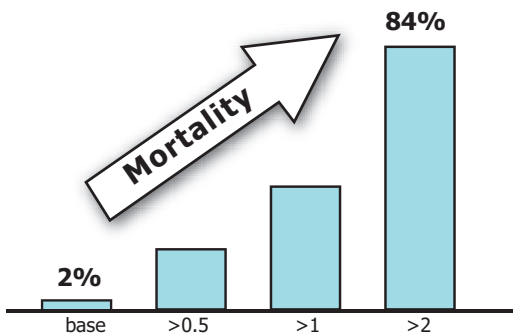
Authors: Suzanne Heemskerk, PhD; Rosalinde Maser-eeuw, PhD; Olof Moesker; Martijn P.W.J.M. Bouw; Johannes G. van der Hoeven, MD, PhD; Wilbert H.M. Peters, PhD; Frans G.M. Russel, PhD; Peter Pickkers, MD, PhD

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Figure 3. Serum creatinine as a marker for kidney function.

Acute Kidney Injury Biomarker

Higher serum creatinine correlates with increased mortality*



Serum creatinine increase in mg/dl over base

*19,982 patients were evaluated on consequences of AKI, Chertow et al, 2005 1 mg/dL serum creatinine = 88µmol/L

AKI can be characterized by elevated serum creatinine levels >150µmol/L (or rapidly deteriorating serum levels). It is known from literature that rapidly increasing and/or high serum creatinine levels are significantly correlated with increased mortality, costs and length of hospital stay (figure 3).

AP has shown to reduce serum creatinine levels in AKI patients after AP therapy (24 hrs infusion).

Human safety studies

Safety was determined in double blind, randomized, placebo controlled studies with bovine intestinal AP. In a first dose escalation study with three groups of eight subjects, 2 of which received matching placebo in each treatment group. Subjects were given a single dose between 7.5 - 67.5 U/kg respectively or matching placebo. A second study was performed with eight subjects who received a dose of 200 U/kg AP or placebo as a continuous infusion up to 72 hours. From the results of the studies, it was concluded that AP was well tolerated. Also, no immunogenic reactions were observed, both in terms of a negative skin prick test (at day 90 post AP administration) and the absence of specific anti-AP antibodies (both IgE and total Ig) up to 90 days after treatment.

MODE OF ACTION

Patients with AKI have reduced AP levels and activity in the kidneys. A short treatment with AP helps restoring renal function in AKI which can be classified as a disease modifying anti-inflammatory effect. The endogenous enzyme alkaline phosphatase (AP) is expressed in various tissues throughout the human body including the kidney. AP is a group of enzymes that maintain homeostasis of the organs in which they are expressed through dephosphorylation of extracellular substrates.

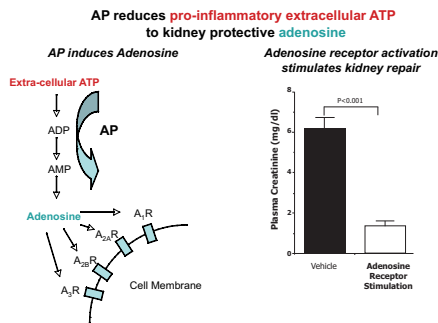
A substrate of AP that can disturb the homeostasis in human organs is adenosine triphosphate (ATP). Dephosphorylation of extracellular ATP has been shown to result in products that restore the homeostasis in the target organs like kidney, by reduction of inflammation induced damage (figure 4). In the normal kidney, inducible glomerular AP acts in the detoxifying process. However, AP can be inactivated by reactive oxygen species and peroxynitrite generated during inflammation, with iNOS expression correlating negatively with AP activity in animal studies.



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Figure 4. The conversion of pro-inflammatory ATP into adenosine and the kidney protective effect of adenosine.

Kidney Repair - via AP induced Adenosine



Trends in Immunology, Vol 25 No 1 January 2004 and Okusa et al. Am. J. Physiol. 277 (Renal Physiol. 46): F404-F412, 1999

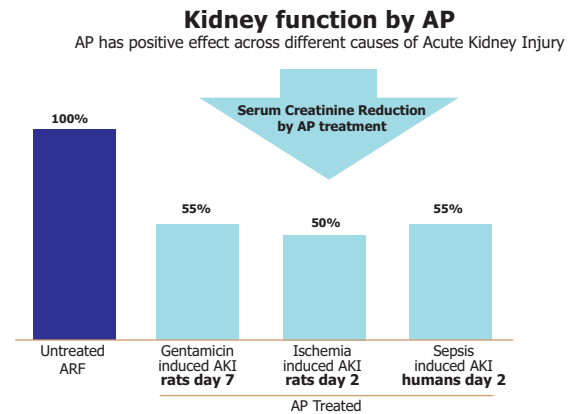
In general extracellular ATP secretion is observed during tissue damage and serves as a danger signal to alert the immune system. ATP is produced by activated polymorphnuclear leukocytes and endothelial cells in inflammation or ischemia. ATP is highly immunostimulatory and activates endothelial cells and immune cells that express the purinergic receptors for ATP to secrete various cytokines, chemokines and upregulates adhesion molecules like ICAM-1. Dephosphorylation of ATP by AP results in the formation of ADP, AMP and adenosine through subsequent dephosphorylation of the different substrates. In contrast to ATP, adenosine has strong anti-inflammatory activity and has been shown to be tissue protective, through adenosine receptor-activation. Especially in the kidney, there is ample evidence that activation of adenosine receptors protects the kidney against ischemia and toxicity-induced renal failure, by prevention of local inflammation.

PRECLINICAL RESULTS

AP improves kidney function in acute kidney injury models

In different rat models the effect of recombinant human AP-treatment on kidney function was examined. The three main causes of acute kidney injury are sepsis, ischemia and toxins. We demonstrated that in all these settings AP-treatment helps to improve kidney function. AP-treatment effects around a 50% reduction of serum creatinine in a short period compared to the AKI-control. Serum creatinine is the key marker to show improvement in kidney function.

Figure 5. AP-treatment has similar positive effect across different causes of Acute Kidney Injury and shows on average a 50% reduction of serum creatinine compared to the control group.



Patent position and recombinant human AP

Proof-Of-Concept results were obtained with an experimental alkaline phosphatase of bovine intestinal origin. AM-Pharma will continue the development program with a recombinant optimized human AP (recAP). The patent for this novel form of AP contains composition of matter claims as well as use and production claims. The new form has excellent stability, activity and significant production benefits. Method of use patents have been filed and granted for AP. Renal failure use patent applications are filed.

Current treatment of Acute Kidney Injury

Current treatment is symptomatic and focuses on correcting fluid and electrolyte balance and uremia. A volume-depleted patient is resuscitated with saline. More often, however, volume overload is present, in which case the following drugs are used: furosemide, intravenously administered calcium, insulin, glucose, inhaled beta agonists or intravenously administered sodium bicarbonate. If these measures do not work, dialysis is initiated. No drugs are approved to treat AKI.

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