Perspectives in Pharmacology

Alkaline Phosphatase as a Treatment of Sepsis-Associated Acute Kidney Injury

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ABSTRACT

Currently there are no pharmacological therapies licensed to treat sepsis-associated acute kidney injury (AKI). Considering the high incidence and mortality of sepsis-associated AKI, there is an urgent medical need to develop effective pharmacological interventions. Two phase II clinical trials recently demonstrated beneficial effects of the enzyme alkaline phosphatase (AP). In critically ill patients with sepsis-associated AKI, treatment with AP reduced the urinary excretion of tubular injury biomarkers and plasma markers of inflammation, which was associated with improvement of renal function. The dephosphorylating enzyme, AP, is endogenously present in the renal proximal tubule apical membrane but becomes depleted during ischemia-induced AKI, thereby possibly contributing to further renal damage. The exact mechanism of action of AP in AKI is unknown, but might be related to detoxification of circulating lipopolysaccharide and other proinflammatory mediators that lose their proinflammatory effects after dephosphorylation. Alternatively, tissue damage associated with systemic inflammation might be attenuated by an AP-mediated effect on adenosine metabolism. Adenosine is a signaling molecule that has been shown to protect the body from inflammation-induced tissue injury, which is derived through dephosphorylation of ATP. In this *Perspectives* article, we discuss the clinical activity of AP and its putative molecular modes of action, and we speculate on its use to treat and possibly prevent sepsis-associated AKI.

Introduction

Acute kidney injury (AKI), a disease characterized by a rapid loss of kidney function, is a common clinical problem in intensive care unit (ICU) patients (Bagshaw et al., 2008b). AKI is a serious condition outside the ICU as well. AKI is diagnosed in up to 20% of hospital admissions and is most often caused by sepsis, cardiac surgery, or nephrotoxic agents (Uchino et al., 2006). Sepsis-associated AKI results in a mortality rate of almost 70%, whereas patients surviving an episode of AKI are at risk of developing chronic kidney disease associated with an enormous financial burden to society (Oppert et al., 2008; Chawla et al., 2011). During sepsis, the initial host response to an infection—mostly caused by Gram-negative or Gram-positive bacteria—becomes amplified and then dysregulated, bringing the body into an inflammatory state (Cohen, 2002).

Nevertheless, therapies aimed to modulate the immune response have led to disappointing results in septic patients (Skirecki et al., 2012).

Currently, supportive renal replacement therapy is the only treatment option available for AKI. Considering the high incidence and related morbidity and mortality of sepsis-associated AKI, there is an urgent medical need to investigate novel pharmacological interventions to treat or prevent AKI.

Pharmacological Interventions

The pathogenesis of AKI in general, and of sepsis-associated AKI in particular, is complex and poorly understood. AKI is thought to be a multifactorial disease with inflammatory, direct nephrotoxic, and ischemic insults acting simultaneously with other pathophysiologic responses to rapidly cause functional failure of the kidney (Bonventre and Yang, 2011; Wen et al., 2011). During sepsis, AKI may be caused by increased renal vascular resistance due to

ABBREVIATIONS: AKI, acute kidney injury; AP, alkaline phosphatase; BiAP, bovine intestinal alkaline phosphatase; ICU, intensive care unit; LPS, lipopolysaccharide; NO, nitric oxide; TLR4, Toll-like receptor 4.

sepsis-induced microvascular alterations or increased intra-abdominal pressure (Bouglé and Duranteau, 2011).

Although several clinical trials to treat AKI have been executed with diverse pharmacological interventions, mostly disappointing results were obtained possibly due to disease heterogeneity and the complex pathogenesis. Recent examples include the use of vitamin E and *N*-acetylcysteine to treat contrast-induced nephropathy, and furosemide treatment of hemofiltration-dependent AKI (van der Voort et al., 2009; ACT Investigators, 2011; Kitzler et al., 2012).

Interestingly, two recent phase II studies demonstrated that parenteral administration of the dephosphorylating enzyme alkaline phosphatase (AP) to ICU patients with sepsis and associated AKI improved kidney function and biomarker readouts of inflammation and kidney injury (Heemskerk et al., 2009; Pickkers et al., 2012). In this *Perspectives* article, we discuss the efficacy of AP as a treatment option for sepsis-associated AKI and its putative modes of action, and speculate on its use to treat and prevent kidney injury.

Clinical Trials

APs are a group of enzymes that exists as four different isoenzymes, which are termed after the tissue in which the enzymes were originally identified, namely placental, germ cell, intestinal, and tissue nonspecific (liver/bone/kidney) AP. The enzyme is also detectable in blood, which represents the amount released by these tissues, and originates predominantly from liver and bone (Moss, 1982). AP was originally considered as a novel treatment of sepsis and was not intended specifically for AKI (Poelstra et al., 1997; Verweij et al., 2004). A variety of animal experiments aimed at obtaining evidence for dephosphorylation and detoxification of the endotoxin lipopolysaccharide (LPS), a component of the outer wall of Gram-negative bacteria that, through the Toll-like receptor 4 (TLR4) pathway, is thought to play a pivotal role during sepsis (Cohen, 2002). The toxicity of LPS is caused by its lipid A moiety, which contains two phosphate groups. Removal of one of these groups by AP results in monophosphoryl-LPS, a far less toxic substance that still binds to TLR4 but predominantly acts as an TLR4 antagonist that prevents the inflammatory response evoked by subsequent exposure to LPS (Bentala et al., 2002). AP was demonstrated to attenuate the immune response by evoking this pathway in mice (Verweij et al., 2004), rats (Poelstra et al., 1997), piglets (Beumer et al., 2003), and sheep (Su et al., 2006).

Initially, clinical development was conducted using purified bovine intestinal alkaline phosphatase (BiAP), a close homolog of human intestinal AP. Preceding the parenteral administration of exogenous BiAP in patients, the clinical pharmacokinetics and safety were assessed (Pickkers et al., 2009). A loading dose of BiAP followed by continuous infusion resulted in relatively stable serum enzyme activity in healthy volunteers, pre-exposed to LPS, as well as in sepsis patients, at a level that is approximately double that of endogenous enzyme for a period of 24–72 hours. This was the basis for developing the infusion scheme as reported, and is in concordance with animal data that described a 2- to 4-fold increase in systemic AP levels in septic sheep treated with BiAP, which was associated with prolonged survival time and decreased circulating interleukin-6 levels compared with untreated sheep (Su et al., 2006). Being a nonsialylated

glycoprotein, BiAP is primarily cleared by the asialoglycoprotein receptor on liver cells; therefore, a loading dose is required to saturate the liver clearance before a steady state of 2-fold higher AP levels than endogenous circulating levels can be reached and maintained (Beumer et al., 2003). In addition, exogenous BiAP was shown not to cause any detectable adverse effects in humans (Pickkers et al., 2009).

From the first phase IIa trial conducted in 36 ICU patients with sepsis, it became clear that BiAP displayed clinical activity, particularly in those patients who also suffered from AKI (Heemskerk et al., 2009). In that trial, sepsis patients with or without AKI were randomized to receive either AP or matching placebo intravenously for 24 hours. BiAP significantly improved kidney function, as was determined by median plasma creatinine levels and attenuated tubular enzymuria. In addition, patients with sepsis, but without signs of AKI during inclusion, appeared less likely to develop AKI after BiAP infusion, according to median plasma creatinine levels. Although the study was not powered for this end point and this result did not reach statistical significance, it does suggest that AP might be of benefit to prevent AKI.

To confirm the promising beneficial renal effects of BiAP, a second prospective phase IIa clinical trial was conducted, which focused specifically on patients with sepsis and evidence of AKI. AKI was defined as a rise in serum creatinine to >150 μmol/l within 48 hours before inclusion and the absence of primary underlying renal disease, or as stage 1 kidney injury or higher according to the Acute Kidney Injury Network creatinine or urine output criteria (Bagshaw et al., 2008a). In this trial, 36 sepsis-induced AKI patients were included, receiving either BiAP or matching placebo intravenously for 48 hours (Pickkers et al., 2012).

BiAP treatment resulted in improvement of renal function as demonstrated by a significantly faster recovery of creatinine clearance, associated with a trend toward reduction in dialysis requirement and duration. The length of ICU stay was also significantly reduced in the patients treated with BiAP compared with the placebo group. Of note, BiAP treatment might have supported improvement of other organs in these critically ill patients according to the Sequential Organ Failure Assessment score, as exemplified by an observed trend toward reduced need for mechanical ventilation. This scoring system can be used to determine organ dysfunction or failure over time and is a good indicator of prognosis (Ferreira et al., 2001). In addition, using a series of urinary biomarkers to assess the course of AKI more acutely and accurately (Vaidya et al., 2008), BiAP infusion was found to induce an almost immediate and clear attenuation of the urinary excretion of two sensitive markers of kidney injury, namely kidney injury molecule-1 and interleukin-18.

Combining the data from both trials ($n = 52$ patients with evidence of AKI) demonstrates that administration of BiAP reduced plasma creatinine levels within 48 hours from a median [interquartile range] of 181 [153–227] to 145 [106–212] μmol/l, compared with a further increase from 180 [152–267] to 216 [113–336] μmol/l in placebo-treated patients ($P = 0.05$). The need for renal replacement therapy in the patients with evidence of AKI also tended to be lower in AP-treated patients: 26% ($n = 27$ AP patients) versus 46% ($n = 24$ placebo patients), ($P = 0.09$, analyzed using Stouffer's method).

Despite the relatively small number of patients included in the two multicenter, double-blind, randomized, placebo-controlled

phase II trials, the consistency of the observed effects, both on biomarkers as well as on renal function, warrants further research and suggests that use of AP as a therapeutic intervention to ameliorate AKI related to sepsis may be favorable.

AP Mode of Action in Sepsis-Induced AKI

Despite these beneficial effects of AP in the treatment of AKI in sepsis patients, the underlying molecular mechanism (s) of AP improving kidney function remain(s) to be unraveled. AP is a membrane-bound endogenously present enzyme that can detoxify molecules through dephosphorylation (Coleman, 1992). There are, indeed, a limited number of candidate substrates potentially connected to inflammation and subsequent kidney damage that could be dephosphorylated by AP. One of these substrates is the endotoxin LPS itself, which elicits potent systemic as well as renal immune activation.

AP-Mediated Detoxification of LPS. The endotoxin LPS is released during a fulminant infection with Gram-negative micro-organisms, or translocates into the systemic circulation directly from the gut after septic shock or an ischemic insult, resulting in secondary endotoxemia. The toxic lipid A part of LPS will bind to LPS-binding protein and, subsequently, to the coreceptor CD14, accessory protein myeloid differentiation protein 2, and the LPS recognition receptor TLR4 present on monocytes, macrophages, and neutrophils. Consequently, the myeloid differentiation primary response gene (MyD88) will be triggered resulting in activation of transcription factor nuclear factor-κB. This establishes an activated inflammatory state characterized by the release of proinflammatory cytokines and acute phase proteins, such as LPS-binding protein and C-reactive protein, as well as enhanced production of various adhesion molecules on leukocytes and endothelium (Cohen, 2002). Besides Gram-negative bacteria, Gram-positive bacteria, and fungal organisms are increasingly common causes of sepsis (Martin et al., 2003). However, the observation that endotoxemia occurs during both Gram-positive and Gram-negative sepsis (Marshall et al., 2004) suggests that translocation of LPS occurs and that the origin of the primary site of infection is of less importance. Therefore, therapies aimed at attenuating LPS-mediated effects may be beneficial in both Gram-negative and Gram-positive sepsis. Indeed, BiAP administration was also associated with improvement of patients with a confirmed Gram-positive infection (Pickkers et al., 2012).

In vitro experiments demonstrated that the dephosphorylating activity of AP inhibited the nuclear factor-κB response to LPS and, as a result, protected against LPS-induced inflammation (Goldberg et al., 2008; Bol-Schoenmakers et al., 2010). In vivo experiments in mice, rats, and piglets showed a reduction in circulating AP levels during an infection, and subsequent restoration of these levels by BiAP administration suppressed the LPS-induced inflammatory response (Bentala et al., 2002; Beumer et al., 2003). Similarly, BiAP infusion attenuated acute inflammation induced by direct injection of *Escherichia coli* into the bloodstream, polymicrobial sepsis caused by cecal ligation and puncture in mice, or by intraperitoneal injection of feces into sheep (Verweij et al., 2004; van Veen et al., 2005; Su et al., 2006).

Data from the safety and pharmacokinetics trial suggested that BiAP infusion did not affect the endotoxin-induced cytokine production in healthy volunteers, possibly due to the large variation in cytokine response and the small groups studied (Pickkers et al., 2009). Because dephosphorylation by AP may take several hours (Koyama et al., 2002), the short duration of endotoxemia in this model (van Deventer et al., 1990) may not be influenced by AP, in contrast to septic patients with persistent endotoxemia in whom AP may be able to dephosphorylate LPS. Indeed, BiAP infusion into sepsis patients with AKI showed that several well established serum markers of systemic inflammatory activity, such as C-reactive protein, interleukin-6, and LPS-binding protein, responded within several hours after treatment and their decrease remained more pronounced compared with the placebo group during the following days (Pickkers et al., 2012). In addition, a reduction in AP levels after cardiac surgery was recently demonstrated to be associated with increased procalcitonin levels and more pronounced hemodynamic instability (Davidson et al., 2012).

Endotoxin-mediated systemic inflammation is thought to be one of the potential causes of AKI, in part through nitric oxide (NO)–mediated systemic vasodilatation followed by renal vasoconstriction with sodium and water retention (Zarjou and Agarwal, 2011). In addition, LPS can act directly on kidney tubule cells expressing TLR4, triggering a local inflammatory response leading to tubulotoxicity (Chowdhury et al., 2006). This receptor localizes to the S1 segment of the proximal tubules and its activation through LPS leads to oxidative stress in downstream S2 and S3 segments (Kalakeche et al., 2011). Normally, tissue nonspecific AP is expressed in the S1, S2, and S3 segments of the proximal tubule apical membrane and intestinal AP in the S3 segment (Pfleiderer et al., 1984; Verpooten et al., 1989). Endogenous glomerular AP levels are upregulated upon an LPS challenge, which may reflect a local host defense mechanism in the kidney during a relatively mild inflammatory insult (Kapojos et al., 2003). However, the renal enzyme activity of endogenous AP declined during a more pronounced insult such as ischemia-induced acute renal failure, a condition that can also be caused by sepsis (Khundmiri et al., 1997). Renal AP depletion may further deteriorate renal function. Therefore, it appears plausible that further kidney damage could be prevented by pharmacological restoration of endogenous AP levels and that this might facilitate renal recovery. Interestingly, treatment with AP in sepsis patients resulted in a reduced stimulation of inducible nitric oxide synthase expression in proximal tubule cells isolated from the urine. Subsequent production of NO metabolites was attenuated and correlated with reduced urinary excretion of the proximal tubule injury marker, glutathione S-transferase A1-1 (Heemskerk et al., 2009). Inducible nitric-oxide synthase is constitutively expressed in the renal proximal tubule and during systemic inflammation its expression is rapidly increased, resulting in excess NO and successive production of harmful peroxynitrite, causing tubular damage (Heemskerk et al., 2006; Fortin et al., 2010).

Detoxification of endotoxin as a potential critical function of AP in AKI is not only relevant for sepsis-associated AKI, but may also be important for treatment of AKI caused by other factors. For instance, it is known that prolonged ischemic conditions in cardiac surgery patients may lead to increased permeability of the gut, predisposing these patients to secondary endotoxemia (Tsunooka et al., 2004). In addition,

other substrate candidates exist, including other pathogen- or danger-associated molecular patterns such as bacterial CpG and flagellin, which have been demonstrated to be detoxified by BiAP (Chen et al., 2010). These bacterially derived inflammation-related products may also be relevant to explain a putative protective and therapeutic effect of AP on gut-associated inflammation (Lukas et al., 2010).

Thus, administering AP during sepsis-associated AKI might both systemically restore and locally supplement endogenous AP levels leading to detoxification of LPS and other bacterial products, and ultimately restore kidney function (Fig. 1). These nonspecific effects of AP may explain why this enzyme might be more effective as an antisepsis therapy compared with therapies solely acting on the LPS-TLR4 pathway, which have not demonstrated to be effective in sepsis patients. For example, the TLR4 antagonist eritoran tetrasodium tended to reduce the mortality rate in a phase II clinical trial with severe sepsis patients at a higher risk of death (Tidswell et al., 2010), but failed to improve 28-day mortality in a subsequent phase III trial (Eisai Co., Ltd., http://www.eisai.com/news/enews201108pdf.pdf). The effects of this compound on renal function are currently not known.

AP-Mediated Dephosphorylation of ATP. ATP is a potent substrate located within the kidney that can also be dephosphorylated by AP. This proinflammatory energy molecule is normally residing within the cell but is released by damaged or inflamed renal tissue and can be dephosphorylated

Fig. 1. Dephosphorylation of LPS by AP. Endotoxin plays an important role in the pathophysiology of sepsis. Binding of LPS to TLR4, expressed on systemic inflammatory cells and renal cells, results in a systemic and renal immune response causing AKI. Removal of one of the two phosphate groups (P) by AP results in a nontoxic reaction product that acts as an antagonist on the LPS receptor TLR4 and reduces the inflammatory response and subsequent renal damage.
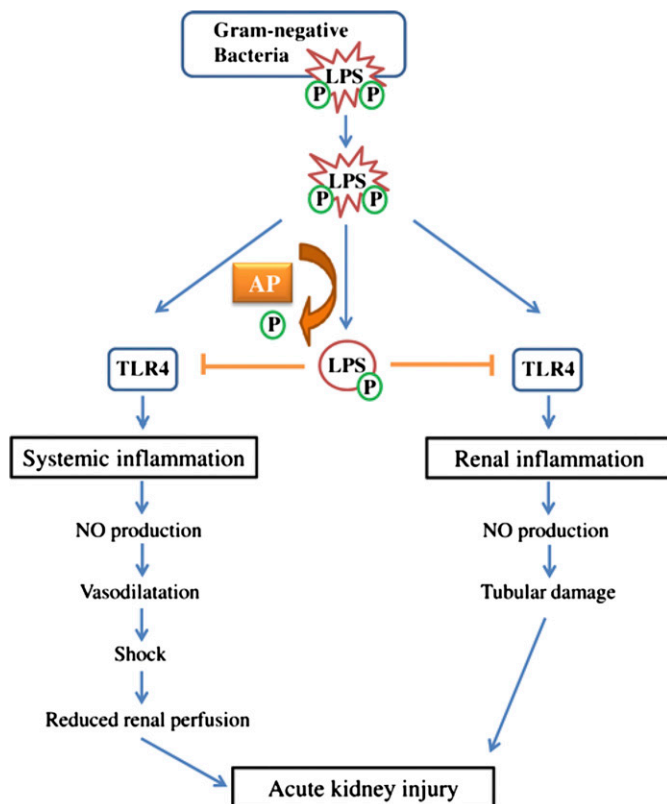
into ADP, AMP, and ultimately into adenosine (Bours et al., 2006). These reactions are catalyzed by a family of enzymes called ectonucleotidases, which are present in all tissues, including the kidney. This family can be classified into four groups based on their distinct hydrolyzing activities: ectonucleoside triphosphate diphosphohydrolase (e.g., CD39), ectonucleotide pyrophosphatase/phosphodiesterase, ecto-5'-nucleotidase (e.g., CD37), and APs (Shirley et al., 2009).

During inflammation, extracellular nucleotide balances may be disturbed (Bours et al., 2006). Therefore, the role of ectonucleotidases in (patho)physiologic processes has been studied extensively; for instance, CD73 has been shown to play a protective role in toxin-induced lung and kidney injury (Volmer et al., 2006; Hasko et al., 2011).
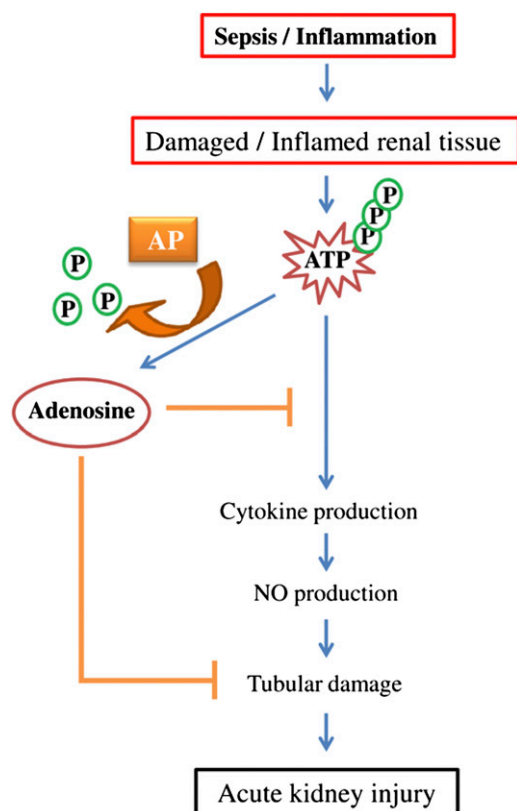
These protective effects are explained by an increased production of adenosine, an anti-inflammatory purine widely produced throughout the body with multiple physiologic functions (Eltzschig, 2009). In the kidney, adenosine is involved in regulating tubular glomerular feedback, renin release, and the glomerular filtration rate (Yap and Lee, 2012) and can bind to one of the four adenosine receptors A1, A2A, A2B, and A3. In stressful circumstances such as systemic inflammation, activation of these four adenosine receptors proved protective against mortality in animal models (Sullivan et al., 2004; Gallos et al., 2005; Lee et al., 2006; Csóka et al., 2010). Adenosine also provides local protection in the kidney during systemic inflammation, via binding to adenosine receptors A1, A2B, and A3 and subsequent downstream signaling (Gallos et al., 2005; Lee et al., 2006; Yang et al., 2006).

Considering these anti-inflammatory effects, local recovery of adenosine levels in the kidney may be beneficial. The close relation between AP and other ectonucleotidases may indicate that the mode of action is shared and encased in the ectonucleotidase activity. Since AP is the only known ectonucleotidase that dephosphorylates ATP and its derivatives into adenosine (Bours et al., 2006), administration of AP may represent an efficient method to restore nucleotide balance in injured tissues with ATP, ADP, and AMP as potent critical substrates, determining the therapeutic effect of AP in AKI patients (Fig. 2).

Putative Therapeutic Use of AP. Both bacterially derived (e.g., LPS) and endogenous (e.g., ATP) substrates are thought to be pivotal targets for the therapeutic activity of AP in sepsis-induced AKI. Whether AP-mediated dephosphorylation of LPS, ATP, and its derivatives, or other substrates are most critical for the beneficial effects of AP is currently unclear. It is known that circulating levels of endogenous AP become depleted during sepsis and that local expression of AP is lost in damaged kidneys (Khundmiri et al., 1997; Bentala et al., 2002). However, it appears unlikely that BiAP infusion simply constitutes replacement therapy in the kidney. For instance, there is no evidence suggesting that therapeutic administration of BiAP leads to its accumulation in the kidney, or that BiAP reaches the proximal tubule apical membrane. The observation that other organs also appear to respond to BiAP infusion could be interpreted as an indication of systemic anti-inflammatory activity and might also support the hypothesis that the kidney is key in organ cross-talk mechanisms (Lukas et al., 2010). Nevertheless, because in septic AKI patients injury biomarkers (kidney injury molecule-1 and interleukin-18) and markers of systemic inflammation (LPS-binding protein, C-reactive protein, interleukin-6) respond within hours

Fig. 2. The proinflammatory molecule ATP is released by damaged or inflamed renal tissue and causes tubular damage and, subsequently, AKI. Dephosphorylation of ATP by AP yields adenosine, which possesses anti-inflammatory properties and prevents tubular damage. Although the role of adenosine in sepsis has, as yet, not been confirmed in humans, this figure illustrates how dephosphorylation of other proinflammatory molecules than LPS by AP potentially may protect the kidneys.

after initiating BiAP treatment, it is likely that its mode of action is both systemic through detoxifying endotoxin and local by neutralizing the proinflammatory activity of extracellular purines and possibly the endotoxin itself.

Future Perspectives

The phase IIa clinical trials demonstrated the potential promising effects of BiAP on kidney function in critically ill patients with sepsis-associated AKI. A novel recombinant human chimeric AP, incorporating the enzyme domain derived from human intestinal AP and the stability domain of human placental AP, is currently under development as a pharmaceutically acceptable alternative replacing purified BiAP (AM-Pharma, http://www.am-pharma.com/products).

Significant effort is made to further elucidate the mode of action of AP in the treatment of kidney injury. Both in vivo and in vitro models may provide better insight into the physiologic response of inflammation or ischemia-induced renal damage, and cell culture systems, such as human proximal tubule epithelial cell lines, may be useful to study the consequences of AP treatment on signaling induced through Toll-like receptor activation, as well as through renal ATP and adenosine receptors. Results of these efforts should provide more definitive proof of the molecular and cellular

mechanism of action of AP in modulating or ameliorating kidney damage within the next few years.

Enhanced understanding of the mode of action of AP will also be important to drive decisions on dosing rationale, dose schedule, and possibly patient selection. Importantly, improved understanding of AP as a treatment of acute kidney damage may not only treat AKI in ICU patients, but might lead to broader use of AP to prevent occurrence of AKI in patients at risk (due to surgery or use of nephrotoxic agents) or to treat failure of other organs, such as the lungs.

Thus far, the data indicate that AP holds a therapeutic promise and distinguishes itself from other therapies, because treatment with the enzyme restores an intrinsic protective mechanism of the human body. Therefore, more extensive research into the molecular mode(s) of action and the efficacy of AP to treat and possibly prevent AKI is clearly warranted, and more clinical data are anticipated with high expectations.

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