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In Translation

Alkaline Phosphatase: A Possible Treatment for Sepsis-Associated Acute Kidney Injury in Critically Ill Patients

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Acute kidney injury (AKI) is a common disease in the intensive care unit and accounts for high morbidity and mortality. Sepsis, the predominant cause of AKI in this setting, involves a complex pathogenesis in which renal inflammation and hypoxia are believed to play an important role. A new therapy should be aimed at targeting both these processes, and the enzyme alkaline phosphatase, with its dual mode of action, might be a promising candidate. First, alkaline phosphatase is able to reduce inflammation through dephosphorylation and thereby detoxification of endotoxin (lipopolysaccharide), which is an important mediator of sepsis. Second, adenosine triphosphate, released during cellular stress caused by inflammation and hypoxia, has detrimental effects but can be converted by alkaline phosphatase into adenosine with anti-inflammatory and tissue-protective effects. These postulated beneficial effects of alkaline phosphatase have been confirmed in animal experiments and two phase 2a clinical trials showing that kidney function improved in critically ill patients with sepsis-associated AKI. Because renal inflammation and hypoxia also are observed commonly in AKI induced by other causes, it would be of interest to investigate the therapeutic effect of alkaline phosphatase in these nephropathies as well.
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INDEX WORDS: Acute kidney injury; alkaline phosphatase; biopharmaceutical; adenosine; sepsis; hypoxia; renal inflammation; renal failure; systemic inflammation.

BACKGROUND

The incidence of acute kidney injury (AKI) in the intensive care unit (ICU) is estimated to be around 20%-50% and contributes to mortality of >50%.[1] Moreover, up to one-third of all critically ill patients surviving an episode of AKI develop chronic kidney disease, accompanied by an enormous financial burden on society.[2] The pathogenesis of AKI is very complex, but >30% of the cases are caused by sepsis.[3] Unfortunately, no pharmacologic interventions currently exist to treat AKI, with only supportive care such as renal replacement therapy (RRT) available. Therefore, an urgent need for new treatment options exists. A promising novel treatment strategy is the enzyme alkaline phosphatase (ALP; also referred to as AP). Originally, ALP was developed as an anti-inflammatory sepsis therapy; however, the enzyme appeared to be predominantly renal protective in an ICU subpopulation of patients with sepsis-associated AKI.[4,5] In this review, we discuss the pathogenesis of sepsis-associated AKI and elaborate on ALP as a possible treatment strategy.

CASE VIGNETTE

A 72-year-old man admitted to the hospital with abdominal pain was suspected to have intestinal ischemia or a ruptured abdominal aneurysm. Antibiotic treatment was started and laparotomy was performed, which revealed intestinal perforation and fecal leakage due to a rectosigmoid mass. Postoperatively, the patient was transferred to the ICU, where antibiotic treatment was continued. Upon admission to the ICU, his blood pressure dropped despite fluid resuscitation (mean arterial pressure < 70 mm Hg), and vasopressor therapy was started. Leukopenia was present (white blood cell count $< 2 \times 10^3/\mu L$). One day later, respiratory insufficiency developed, ventilatory support was initiated, and blood cultures grew Gram-negative rods. The following day, urine flow decreased to <0.5 mL/kg/h, and the patient's serum creatinine level increased from 1.14 mg/dL (101 μmol/L; corresponding to creatinine clearance [CCr] by the Cockcroft-Gault equation of 83 mL/min) to 1.44 mg/dL (128 μmol/L; CCr, 65 mL/min).[6]

The patient fulfilled the criteria of a phase 2a clinical trial of ALP[5] and was enrolled in the study. Upon deblinding at the end of the trial, it was revealed that he had been randomly assigned to the group receiving ALP. During the 24 hours prior to ALP administration, his urine flow was 0.5 ± 0.1 mL/kg/h, but for the 48 hours following drug treatment, it increased to 0.7 ± 0.3 mL/kg/h. Simultaneously, his creatinine level decreased to 1.29 mg/dL (114 μmol/L; CCr, 106 mL/min). The following day, the patient underwent a second laparotomy and proceeded to recover quickly. While the clinical course was complicated by a wound dehiscence for which additional surgery was required and an episode of delirium, kidney function improved over the next days as his creatinine level decreased to

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0.95 mg/dL (84 μmol/L; CCr, 100 mL/min) and urine flow increased to 1.4 ± 0.7 mL/kg/h. The patient was extubated on day 7 and discharged from the ICU the following day. During study follow-up at day 28, serum creatinine level decreased further to 0.61 mg/dL (54 μmol/L; CCr, 155 mL/min).

PATHOGENESIS

The pathogenesis of sepsis-associated AKI is far from completely understood due to its complexity and multifactorial origin and because of the lack of kidney biopsies from this vulnerable patient population. The concept of reduced renal blood flow as a sole contributor to sepsis-associated AKI currently is questioned because several studies of animals and of humans have shown that sepsis-associated AKI develops during normal or even increased renal blood flow.[7] These findings introduce a paradigm shift, suggesting an important role for inflammation, altered renal microcirculation, and possibly unbalanced renal bioenergetics (Fig 1).[8]

Inflammatory Response

During sepsis, the body's response to an infection is characterized by the release of several detrimental inflammatory mediators, including many proinflammatory cytokines and arachidonic acid metabolites; upregulation of inducible nitric oxide (NO) synthase (iNOS); and activation of the complement cascade.[9] Another harmful mediator is extracellular adenosine triphosphate (ATP), which is released during cell stress and stimulates inflammation and tissue injury by attracting phagocytes and activating the NLRP3 inflammasome.[10,11] Sepsis can be caused by different pathogens, and certain motifs of these pathogens, known as pathogen-associated molecular patterns, are recognized by the innate immune system. These pathogen-associated molecular patterns signal by Toll-like receptors (TLRs) or other pathogen recognition receptors, thereby triggering an inflammatory response.[12]

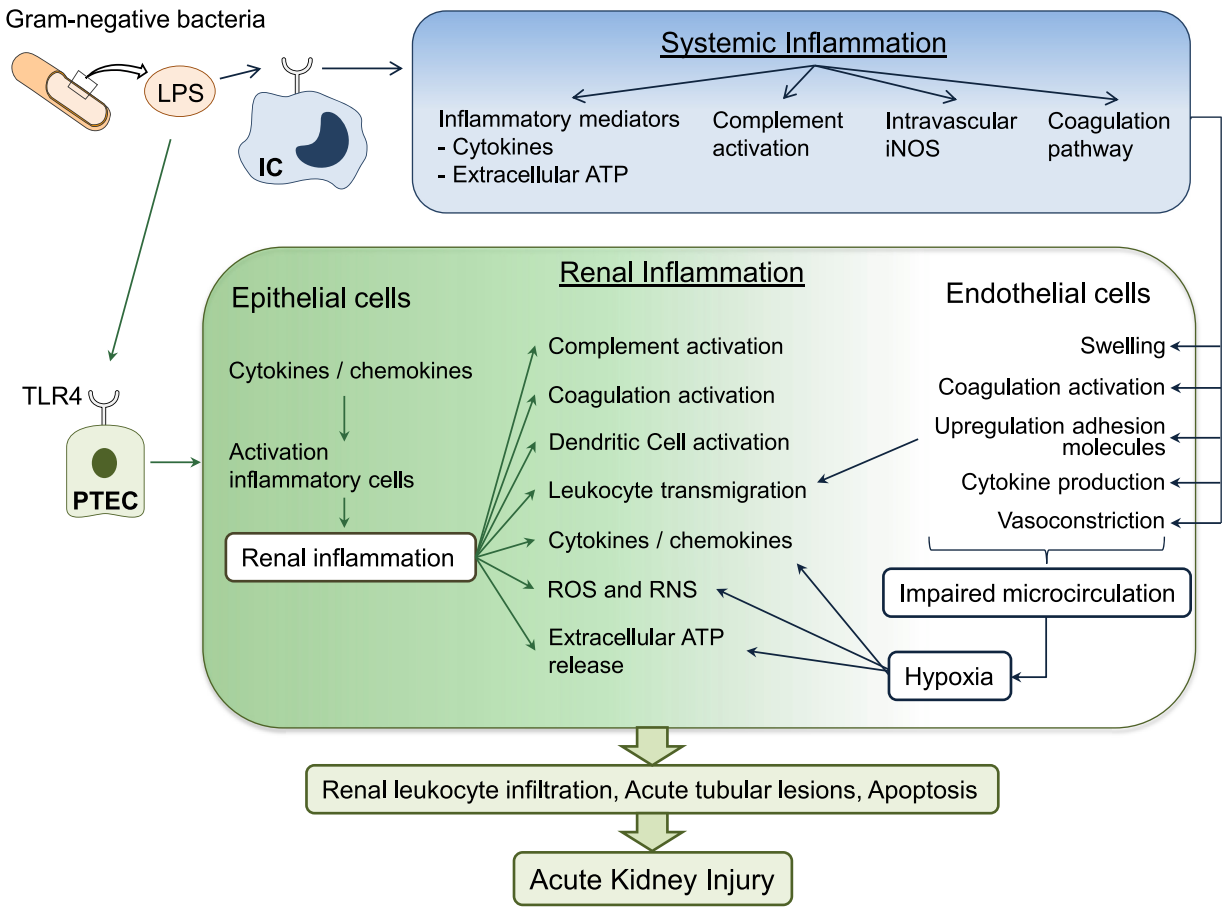

Figure 1. Renal inflammation and impaired microcirculation in the pathogenesis of sepsis-associated acute kidney injury (AKI). Lipopolysaccharide (LPS) binds to Toll-like receptor 4 (TLR4) on immune cells (ICs), triggering the inflammatory response causing systemic inflammation. Alternatively, LPS binds to TLR4 expressed on proximal tubule epithelial cells (PTECs), inducing renal inflammation. Endothelial cells exposed to LPS and circulating cytokines may result in impaired renal microcirculation, causing hypoxia. The immune response is enhanced further by renal inflammation and hypoxia, eventually causing AKI characterized by leukocyte infiltration, acute tubular lesions, and apoptosis. Abbreviations: ATP, adenosine triphosphate; iNOS, inducible nitric oxide synthase; ROS, reactive oxygen species; RNS, reactive nitrogen species.

In critically ill patients, bacterial isolates are predominantly Gram-negative.[13] The outer membrane of Gram-negative bacteria is made up in part of lipopolysaccharide (LPS), which is the most thoroughly studied endotoxin and is considered an important pathogen-associated molecular pattern causing sepsis through TLR4 signaling.[14] LPS binds in complex with LPS-binding protein (LBP) to TLR4. Subsequently, the transcription factor NF-κB (nuclear factor-κB) is activated through myeloid differentiation primary response gene 88 (MyD88)-dependent and -independent pathways, resulting in the activation of cytokine gene promoters.[15] This results in the systemic release of pro- and anti-inflammatory cytokines, further enhancing hematopoiesis, phagocytosis, and recruitment of leukocytes toward the site of inflammation.[16]

Interactions between LPS and TLR4 also can occur locally in the kidney, resulting in activation of the NF-κB signaling pathway. TLR4 is endogenously expressed in the cortex and medulla and appears to be upregulated in the renal tubules, glomeruli, and renal vasculature during polymicrobial sepsis.[17] Within 10 minutes after LPS infusion in mice, the endotoxin is present in renal tissue, where it interacts with TLR4 localized at the brush border membrane of the first part of the proximal tubule.[18] This results in downstream oxidative stress in lower segments of these tubules.[19]

Aside from triggering oxidative stress, LPS activates the immune system, resulting in a local inflammatory response in the kidney.[20,21] Tubular epithelium in the outer medullary region responds to LPS by producing proinflammatory and chemotactic cytokines, such as tumor necrosis factor α (TNF-α), MCP-1 (monocyte chemoattractant protein 1), and interleukin 6 (IL-6) and IL-8, which will result in the activation of inflammatory cells.[22] This is accompanied by complement and coagulation pathways activation, protease activation, production of reactive oxygen species (ROS) and nitrogen species (RNS), and upregulation of TLR4,[22] further accelerating the inflammatory cascade through resident dendritic cells.[23] Subsequently, these cells produce several proinflammatory cytokines and attract macrophages, neutrophils, and other immune cells as well.[23] This results in morphologic and functional changes in epithelial and endothelial cells, for example, loss of the brush border membrane and formation of vacuoles,[24] activation of Fas- and caspase-mediated pathways,[25] and nuclear condensation,[26] causing the development of tubular apoptosis. Both cell death and renal leukocyte infiltration are, together with acute tubular lesions, important events in the pathogenesis of sepsis-associated AKI.[27] These findings illustrate that inflammation by itself may account for an important part of the AKI observed in patients with sepsis (Fig 1).

Renal Microcirculation

The renal vascular endothelium also will be exposed directly to pathogens and inflammatory mediators (eg, proinflammatory cytokines), triggering the upregulation of endothelial adhesion molecules and production of cytokines and chemokines. This results in endothelial-leukocyte interactions causing leukocyte transmigration toward renal interstitium, further enhancing epithelial inflammation through the production of cytokines, ROS, and RNS.[20] Sepsis-induced endothelial leukocyte transmigration, together with the activated coagulation system, endothelial swelling, and enhanced vasoconstriction of the arterioles, results in a compromised renal microcirculation.[28,29] The impaired renal microcirculation, another important feature in the pathogenesis of sepsis-associated AKI, causes heterogeneity of blood flow, resulting in some parts of the kidney being well perfused and other parts being hypoperfused and hypoxic.[30,31] Renal hypoxia is harmful for the kidney because it affects the balance of ROS, NO production, and renal oxygenation. Inflammation increases iNOS activation in the kidney, resulting in the production of an excessive amount of NO. Subsequently, NO reacts with superoxide ions, leading to the generation of RNS such as peroxynitrite, and both nitrosative and oxidative stress further compromise the microcirculation.[32]

In addition, hypoxia activates several protective genes, such as hypoxia-inducible factor (HIF), which plays a major role in the body's adaptation to hypoxia through the extracellular formation of adenosine.[33,34] The endogenous signaling molecule adenosine reduces several harmful effects of hypoxia, including hypoxia-induced inflammation, ischemia-associated organ dysfunction, and hypoxia-induced vascular leakage.[35] Adenosine is present ubiquitously, including in the kidney, where it is involved in the regulation of blood flow by tubuloglomerular feedback mechanisms, glomerular filtration rate, renin release, and vascular tone.[36] Adenosine signals through binding to 1 of the 4 adenosine receptors, A1, A2A, A2B, and A3, all of which are expressed on immune cells[37] and nephron segments, including the glomerular epithelium, renal vasculature, proximal tubules, and collecting ducts.[38] Extracellular adenosine is derived mainly from the phosphohydrolysis of ATP, adenosine diphosphate (ADP), and adenosine monophosphate (AMP) or through conversion of cyclic AMP. These reactions are all catalyzed by enzymes belonging to the family of ectonucleotidases.[39,40] HIF increases extracellular levels of adenosine through transcriptional induction of ectonucleotidases and attenuation of adenosine breakdown enzymes and uptake transporters and increases

signaling through enhancement of adenosine A2B receptor expression.[34,35] Adenosine receptor binding on inflammatory cells increases intracellular second-messenger cyclic AMP levels, resulting in activation of several anti-inflammatory pathways, causing leukocyte inhibition.[36,41]

In the kidney, HIF is widely expressed and detected in renal epithelial cells, endothelial cells, and renal interstitial fibroblast-like cells.[33,42,43] During hypoxic circumstances, HIF appears to protect these cells by reducing oxidative stress and increasing cell survival.[33,42,43] The observations that detrimental ATP is released from inflammatory or apoptotic cells during circumstances of limited oxygen availability and inflammation[44-47] and that intrarenal adenosine concentrations are increased during hypoxia[48] strongly suggest that these HIF-induced effects are mediated through extracellular adenosine as well (Fig 2). In addition, adenosine protects human proximal tubular epithelial cells from oxidant injury by adenosine receptors.[49] Together, this indicates an important contribution of impaired renal microcirculation to the development of sepsis-associated AKI by causing renal hypoxia, against which the kidney might protect itself by elevated levels of extracellular adenosine.

Renal Bioenergetics

Another pathophysiologic process that potentially is relevant in the development of sepsis-associated AKI is represented by an imbalance in renal bioenergetics. Mitochondrial dysfunction plays a major role in the development of multiorgan failure during sepsis. Several mediators present during septic shock, including peroxynitrite, can inhibit the mitochondrial respiratory chain and decrease oxygen consumption.[50] Mitochondrial respiration during prolonged endotoxemia is impaired in some organs, such as the liver, but appears to be unaffected in renal tissue.[51] Recently, these findings were confirmed in a sheep model system of hypotensive sepsis, in which unaltered renal bioenergetics were observed in the early phase of sepsis-associated AKI.[52] However, despite the lack of depletion of renal energy, ATP consumption can still be compromised.[53] Because the role of renal bioenergetics in the development of sepsis-associated AKI is not completely elucidated to date, more research needs to be done to fully understand its involvement in the pathogenesis.

In summary, sepsis-associated AKI may develop as a result of an exacerbated inflammatory response, systemically and locally within the kidney, and an altered renal microcirculation, resulting in renal hypoxia. Both hypoxia and the inflammatory response induce the release of several harmful mediators (eg, cytokines) and cause nitrosative and oxidative stress, provoking injury to renal epithelium and endothelium (see Fig 1). In order to prevent the development of sepsis-associated AKI, a therapy targeting both the immune response and hypoxia-driven injury might be beneficial to the kidney.

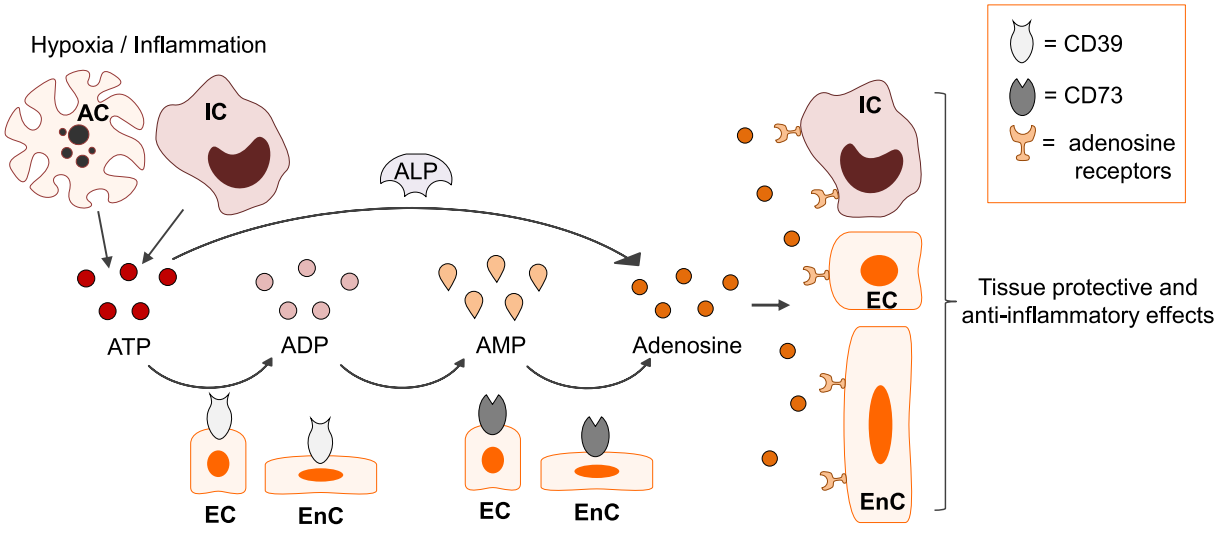

Figure 2. Detrimental extracellular adenosine triphosphate (ATP) may be converted by alkaline phosphatase (ALP) into tissue-protective and anti-inflammatory adenosine. During hypoxia and inflammation, ATP is released from inflammatory (ICs) and apoptotic cells (ACs), which can be converted by CD39 and CD73 expressed on renal endothelial (EnC) and epithelial cells (ECs) into adenosine diphosphate (ADP), adenosine monophosphate (AMP), and subsequently adenosine. Exogenously present ALP also may convert ATP into adenosine, which then can bind to adenosine receptors on EnCs, ECs, and ICs, resulting in renal-protective and anti-inflammatory effects. Abbreviation: CD, cluster of differentiation.

RECENT ADVANCES

Overview

Currently, it is not clear whether measures aimed at preventing AKI in patients with sepsis, including volume expansion and possibly the use of diuretics, vasopressors, and inotropes, are effective.[54] However, once AKI develops, only general supportive measures are available, such as lung-protective ventilation and RRT.[54] Despite numerous attempts to find new therapies for sepsis-associated AKI, there is still no pharmacologic treatment available because several promising therapies failed when tested in clinical trials.[55-57] This might be explained by the heterogeneity of the study populations in comparison to animal studies, the complex pathogenesis of sepsis-associated AKI, and because the disease affects multiple organ systems.[58]

The multifactorial pathogenetic response to both inflammation and hypoxia necessitates a multifactorial intervention to allow protection against sepsis-associated AKI. Two phase 2a clinical trials suggest ALP as a novel promising anti-inflammatory biologic with such a dual mechanism of action (Fig 3).[4,5] ALP originally was developed as an anti-inflammatory sepsis drug because it dephosphorylates and detoxifies LPS,[59] a key player in sepsis pathogenesis.[14]

Characteristics and Functions of ALP

ALP is a membrane-bound homodimeric enzyme that contains 2 zinc ions and one magnesium ion per active site, which are necessary for its activity.[60] This dephosphorylating glycoprotein is most stable in the pH range of 7.4-9.8[61] and catalyzes transphosphorylation reactions and the hydrolysis of monoesters, thiophosphates, phosphorothiates, and phosphoramidates (eg, ATP).[60]

Four different isoenzymes are known, namely placental, germ cell, intestinal, and tissue-nonspecific (liver/bone/kidney) ALP (also known as PLAP, GCAP, IAP, and TNAP, respectively), which illustrates the expression of ALP and its broad array of functions during health and disease.[62] ALP measured in serum is derived mostly from liver and bone.[63] The function of ALP in the intestine has been studied extensively in animal studies; intestinal ALP has been shown to be involved in the regulation of pH and bicarbonate secretion, regulation of lipid absorption across the enterocyte apical membrane, and detoxification of bacterial endotoxins through dephosphorylation.[64] Bone ALP is suggested to be involved in bone mineralization, but the exact role of the other isoenzymes is still unknown.[62] However, they are implicated as markers of disease because elevated serum levels of placental ALP, bone-derived ALP, and liver-derived ALP can be used as markers for testicular cancer,[65] fracture prediction,[66] and cholestasis,[67] respectively.

In the kidney, 2 different iso-enzymes are expressed on the brush border membrane of the proximal tubule cell: tissue-nonspecific ALP is present in the entire proximal tubule and intestinal ALP is localized in the lower tubular segments.[68-70] As with other organs, little is known about the function of ALP in the kidney, but after LPS administration to rats, a reduction in kidney function and loss of brush border membranes are observed, accompanied by increased ALP levels in urine.[24] Additionally, during renal ischemia-reperfusion injury, it has been shown that the ALP brush border activity, measured in vesicles isolated from whole cortex, is attenuated while ALP levels are increased in urine, indicating injury to the proximal tubule brush border.[71,72]

A Protective Effect of ALP in Sepsis-Associated AKI?

Renal inflammatory injury and hypoxia, caused by impaired renal microcirculation, appear to represent key players in the pathogenesis of sepsis-associated AKI. In order to find a treatment strategy for this disease, new therapies should target both renal inflammation and renal hypoxia, and the enzyme ALP is a promising biologic agent.

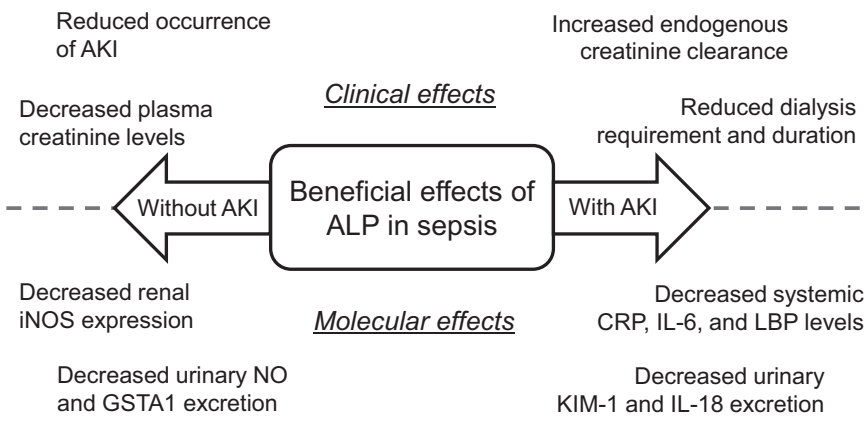

Figure 3. The beneficial clinical and molecular effects of alkaline phosphatase (ALP) administration to critically ill patients with severe sepsis/septic shock, with or without acute kidney injury (AKI). Abbreviations: CRP, C-reactive protein; GSTA1, gluthathione-*S*-transferase A1; IL, interleukin; iNOS, inducible nitric oxide synthase; KIM-1, kidney injury molecule 1; LBP, lipopolysaccharide-binding protein; NO, nitric oxide.

LPS as a Therapeutic Target

In view of the detrimental role of LPS in the development of sepsis, endotoxin might represent an interesting pharmacologic target. Unfortunately, the direct targeting of LPS by an immunoglobulin G anti-LPS antibody,[73] as well as inhibition of the LPS signaling pathway through TLR4 antagonism by Eritoran,[74] have been found ineffective in ICU patients with severe sepsis. Another strategy could be the detoxification of LPS. LPS is composed of a polysaccharide chain, an oligosaccharide core component, and the lipid A part, the latter causing the toxicity of this molecule. This toxic moiety is composed of 2 carbohydrate units and several fatty acid chains. Each carbohydrate usually contains one phosphate group, and removal of the phosphate groups on one of the carbohydrate units by a dephosphorylating molecule results in a nontoxic lipid A moiety.[59] Dephosphorylated LPS is far less toxic, binding to TLR4 but failing to activate it, and thus working as a TLR4 antagonist.

According to several in vitro and in vivo experiments, LPS can be dephosphorylated by ALP. In rats, inhibition of endogenous ALP activity by administration of levamisole significantly decreases survival rates of animals exposed to a sublethal dose of Gram-negative *Escherichia coli,* whereas not affecting survival of animals given the Gram-positive bacterium *Staphylococcus aureus.*[75] In several animal models, exogenous placental ALP and bovine intestinal ALP have been shown to attenuate the inflammatory response induced by LPS and improve survival.[59,76] In mice, placental and bovine intestinal ALP appear to be protective during *Escherichia coli*–induced inflammation because treatment reduces fever and attenuates the systemic cytokine response, serum NO levels, and liver and lung damage.[77,78] Also, treatment with bovine intestinal ALP preceding the administration of LPS results in an accelerated recovery according to physiologic parameters (eg, temperature and heart and breathing rates), reduced TNF-α response, and attenuated hematologic changes in piglets.[76] Furthermore, intestinal ALP reduces blood IL-6 concentration and improves gas exchange during septic shock induced by fecal peritonitis in sheep.[79] These beneficial effects are accompanied by an ALP-mediated reduction in serum LPS levels because it has been shown that serum LPS levels significantly increase in the presence of the intestinal ALP inhibitor L-phenylalanine.[80] ALP seems to interact with the LPS-TLR4 pathway because the LPS-induced increase in NF-κB activity is attenuated in endothelial cells pretreated with exogenous bovine intestinal ALP. Also, upregulation of endogenous intestinal ALP by the proinflammatory Resolvin-E1 reduces LPS-induced NF-κB activity.[81] This reduction probably is mediated through dephosphorylation and thereby detoxification of LPS. The ability of ALP to dephosphorylate LPS has been demonstrated through the presence of free phosphate using enzymatic in vitro assays and in vivo histochemical analysis, revealing phosphatase activity present at the tubular brush borders.[59,75,77] Together, these studies have demonstrated several anti-inflammatory effects of ALP on LPS-induced systemic inflammation (Table 1).

Unfortunately, kidney function was not assessed in these models and the effects of ALP in this respect were not investigated. Because systemic inflammation directly affects renal microvascular blood flow and improvement in renal microcirculation protects the tubular epithelium and prolongs survival in a sepsis-induced AKI mouse model,[82] the effects of ALP on LPS-induced inflammation also may be renal protective. Attenuating circulating LPS and cytokine levels by ALP could have the same beneficial effects, thereby preventing the development of renal hypoxia and subsequent AKI. Furthermore, the detoxification of LPS by ALP locally within the kidney may exert direct anti-inflammatory effects and potentially could prevent the development of AKI. Because LPS causes loss of the proximal tubule brush border membrane, thereby depleting renal ALP,[24] local restoration of the enzyme levels by ALP supplementation might be an evident solution to prevent kidney injury.

ATP as a Therapeutic Target

Another protective compound during sepsis-associated AKI could be extracellular adenosine, which can reduce several harmful effects of hypoxia.[34] Adenosine also can protect the body from injury caused by inflammation. Administering a synthetic adenosine analogue has been observed to reduce circulating TNF-α levels and improve survival in mice receiving a normally lethal dose of LPS,[83] whereas increased survival also has been observed in endotoxemic mice treated with an adenosine A2A receptor agonist.[84] Pretreatment of healthy volunteers with dipyridamole, which increases extracellular adenosine concentrations by blocking its cellular uptake, results in a more rapid decline in levels of the proinflammatory cytokines IL-6 and TNF-α and enhances the LPS-induced increase in the anti-inflammatory cytokine IL-10 during experimental endotoxemia.[85]

The immune-modulating effects of extracellular adenosine also may protect the kidney because activation of the adenosine receptors A2A and A2B in the tubular lumen exerts anti-inflammatory effects.[36] Mice lacking either adenosine receptor A1 or A3 show increased renal expression of proinflammatory

Table 1. Reported Beneficial Effects of ALP Administration in Preclinical Studies of Systemic Inflammation

Study	Species	Model	Treatment	Sample Size[a]	Outcome
Bentala et al[59] (2002)	Mice	Intraperitoneal D-galactosamine and LPS injection	IV bolus injection of 0.1 U placental ALP	7	Survival rate: 57% (untreated) vs 100% (treated), P = NR
Verweij et al[77] (2004)	Mice	Intraperitoneal *E coli* injection	IV bolus injection of 1.5 U placental ALP	14	Survival rate: 58% (untreated) vs 100% (treated), $P < 0.01$ Serum NO levels[b]: 75 μmol/L (untreated[c]) vs 16 μmol/L (control [no *E coli*][d]), $P < 0.01$; 39 μmol/L (treated) vs 16 μmol/L (control [no *E coli*][d]), $P > 0.05$
Beumer et al[76] (2003)	Mice	Intraperitoneal *E coli* injection	IV bolus injection of 1.5 U bovine intestinal ALP	5	Survival rate: 20% (untreated) vs 80% (treated), $P < 0.01$ Body temperature: 34.2°C ± 0.8°C (untreated) vs 36.2°C ± 0.7°C (treated), $P < 0.05$
van Veen et al[78] (2005)	Mice	Cecal ligation and puncture	IV bolus injection of 0.15 U/g body weight bovine intestinal ALP	8	Plasma TNF-α peak level: 170 pg/mL (untreated) vs 57.5 pg/mL (treated), $P < 0.05$ Plasma IL-6 peak level: 19.3 pg/mL (untreated) vs 3.4 pg/mL (treated), $P < 0.05$ Plasma MCP-1 peak level: 2.0 pg/mL (untreated) vs 1.0 pg/mL (treated), $P < 0.05$ Liver damage (AST level[b]): 1,300 IU/L (untreated) vs 800 IU/L (treated), $P < 0.05$ Lung damage (MPO activity[b]): 137.5 U/g (untreated) vs 90 U/g (treated), $P < 0.05$
Koyama et al[80] (2002)	Rats	Oral LPS administration	Oral administration of 40 mg/kg body weight L-phenylalanine (inhibitor of intestinal ALP)	3	Serum LPS level[b]: 340 pg/mL (untreated) vs 180 pg/mL (treated), $P < 0.05$
Su et al[79] (2006)	Sheep	Intraperitoneal feces injection	Intestinal ALP: IV bolus injection of 60 U/kg body weight, then continuous infusion of 20 U/kg/h for 15 h	NR	Plasma IL-6 level[b]: 0.20 AU (untreated) vs 0.16 AU (treated), $P < 0.05$ Gas exchange (Pao_2:Fio_2 ratio)[b]: 50 mm Hg (untreated) vs 320 mm Hg (treated), $P < 0.05$
Beumer et al[76] (2003)	Piglets	IV LPS injection	IV injection of 2,500 U bovine intestinal ALP	NR	Thrombocyte counts: 19 ± 1 × 10^9/L (untreated) vs 41 ± 4 × 10^9/L (treated), $P < 0.05$

Abbreviations: ALP, alkaline phosphatase; AST, aspartate aminotransferase; AU, arbitrary units; *E coli*, *Escherichia coli*; Fio_2, fraction of inspired oxygen; IL-6, interleukin 6; IU, international units; IV, intravenous; LPS, lipopolysaccharide; MCP-1, monocyte chemoattractant protein 1; MPO, myeloperoxidase; NO, nitric oxide; NR, not reported, TNF-α, tumor necrosis factor α.

[a]Number of animals per group, unless otherwise noted.

[b]Values are estimated from a graph.

[c]Only 8 animals survived to have serum NO measured.

[d]There were 6 animals in the control group.

markers and ICAM-1 (intracellular adhesion molecule 1) and enhanced infiltration of leukocytes in septic peritonitis.[86,87] Adenosine is derived mainly from dephosphorylation of ATP. In the kidney, the most essential enzymes involved in these reactions are the ectonucleotidases CD39 (converting ATP into both ADP and AMP) and CD73 (converting AMP into adenosine).[40] Their importance has been demonstrated in vivo because depletion of CD73 increases kidney injury following sepsis.[88] As extracellular ATP is released during hypoxia and inflammation by inflammatory and apoptotic cells,[44-47] and considering its harmful effects, rapid hydrolysis of ATP into adenosine clearly is warranted. Intriguingly, ALPs are the only ectonucleotidases known to be able to fully degrade extracellular ATP into adenosine. Because ALP is depleted from the proximal tubule during AKI, administration of exogenous ALP could result in increased turnover of ATP into adenosine within the kidney.[40] Subsequently, adenosine can exert its anti-inflammatory and tissue-protective effects during hypoxia and inflammation, thereby preventing the

development of sepsis-associated AKI and/or attenuating its persistence (Fig 2).

Clinical Trials and Future Potential Therapeutic Options

In sum, administering exogenous ALP could prevent the development of sepsis-associated AKI, either through dephosphorylation and detoxification of LPS and/or by increasing extracellular adenosine by dephosphorylation of extracellular ATP. The enhancement of ATP turnover into adenosine by ALP might be the most important mechanism because this may cause a switch of the body's proinflammatory condition toward a more anti-inflammatory state, thereby directly targeting the ongoing inflammatory processes.

Before ALP was administered to patients, the clinical pharmacology and safety of bovine intestinal ALP was confirmed in a pharmacokinetic and pharmacodynamic study.[89] During the first phase 2a clinical trial conducted with patients with severe sepsis or septic shock admitted to the ICU (with or without AKI), treatment with bovine intestinal ALP appeared to improve kidney function because patients with AKI showed a reduced need for RRT during the 28-day follow-up period, whereas patients without AKI during enrollment appeared to be less prone to the development of AKI.[4] However, because of the small number of patients and heterogeneity within the study group, these clinically relevant effects did not reach statistical significance. In addition and in line with these clinical end points, bovine intestinal ALP treatment was observed to attenuate the upregulation of renal iNOS expression, resulting in a reduction in urinary NO metabolites, and reduce urinary excretion of the proximal tubule injury marker glutathione-*S*-transferase A1 (GSTA1).[4]

Considering these promising effects, a second phase 2a clinical trial was designed to focus on patients with severe sepsis or septic shock with evidence of early AKI.[5] Treatment with bovine intestinal ALP improved overall kidney function with respect to endogenous CCr, need for RRT, and duration of RRT. Also, the decline in levels of the inflammatory markers C-reactive protein, LBP, and IL-6 was more pronounced in the treatment group compared to placebo, as was the reduction in urinary excretion of the renal damage markers kidney injury molecule 1 (KIM-1) and IL-18.[5]

In light of these results (Fig 3), ALP can be considered as a potential new drug therapy for sepsis-associated AKI. In contrast to many treatment strategies that failed in clinical phases, this treatment encompasses a dual mechanism of action, which makes it an interesting new treatment option. Because ALP treatment appeared to exert beneficial effects on kidney function in patients with sepsis without AKI, a pre-emptive role of ALP against the development of AKI could be considered as well. Importantly, results need to be reconfirmed in larger clinical trials because the 2 initial clinical studies were conducted with a small number of patients.

Considering the postulated dual mechanism of action of ALP influencing inflammatory and hypoxic aspects of sepsis-associated AKI, ALP also could be considered as a treatment option for AKI induced by other causes, such as trauma or cardiac surgery, in which these key pathophysiologic events also are involved. During trauma, ~40% of patients develop AKI, which is caused mostly by secondary sepsis (52%) and hypotension (34%).[90] In cardiac surgery, nearly one-third of all patients develop AKI, which is a serious complication of cardiopulmonary bypass. Cardiopulmonary bypass is believed to cause systemic inflammation characterized by the activation of vascular endothelium, production of cytokines and chemokines, and activation of complement and coagulation systems.[91] These processes potentially could be targeted by ALP treatment.

Currently, a novel recombinant human chimeric ALP is under development, containing the stability domain of human placental ALP and the enzyme domain derived from human intestinal ALP, which would be a pharmaceutically acceptable alternative to replace the bovine form of intestinal ALP.[92] The safety and tolerability of chimeric ALP in humans currently is under investigation in a phase 1 clinical trial.[93] Interestingly, preliminary data indicate that this chimeric protein almost completely attenuates the LPS-induced cytokine response of TNF-α, IL-6, and IL-8 in a human renal cell line (unpublished observations, E. Peters, August 2013).

Nonrenal Effects of ALP

Treatment of patients with sepsis-associated AKI with bovine intestinal ALP has been observed to significantly reduce the duration of mechanical ventilation,[5,94] an interesting finding because lung epithelium directly exposed to LPS and local inflammation can cause extravasation of neutrophils and other inflammatory cells.[95] In other work, pretreatment of mice exposed to experimental autoimmune encephalomyelitis with bovine intestinal ALP has been shown to reduce the neurologic signs present during this neuroinflammatory condition.[96] Furthermore, animal models of necrotizing enterocolitis have demonstrated that bovine intestinal ALP treatment decreases intestinal injury, nitrosative stress, and the associated systemic inflammatory response resulting from this gastrointestinal disease.[97-99] Moreover, beneficial effects of bovine intestinal ALP treatment have been observed in patients with moderate to severe ulcerative colitis.[100] These findings support a broader application of ALP, but these nonrenal effects are beyond the scope of this review.

SUMMARY

Sepsis-associated AKI is accompanied by high morbidity and mortality, and a new pharmacologic treatment option clearly is warranted. ALP seems to be a promising new therapy for patients with sepsis-associated AKI because it restores an endogenously expressed protective enzyme that appears to exert a dual mechanism of action. ALP may prevent the effects of inflammation and hypoxia, key events in the pathogenesis of sepsis-associated AKI, through dephosphorylation of LPS and extracellular ATP, the latter being converted to adenosine, an anti-inflammatory and tissue-protective signaling molecule. Therefore, ALP seems to be a potent new treatment option for patients with sepsis-associated AKI and demands the execution of larger clinical trials. Because inflammation and hypoxia are also involved in AKI induced by several other causes, including cardiac surgery and trauma, it would be of interest to also further examine the therapeutic effects of ALP in these clinical circumstances.

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