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

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Key Words

Alkaline phosphatase · Sepsis · Acute kidney injury · Renal inflammation · Systemic inflammation · Lipopolysaccharide · Adenosine

Abstract

Sepsis-associated acute kidney injury (AKI) is associated with a high attributable mortality and an increased risk of developing chronic kidney failure in survivors. As a successful therapy is, as yet, unavailable, a pharmacological treatment option is clearly warranted. Recently, two small phase II clinical trials demonstrated beneficial renal effects of bovine-derived alkaline phosphatase administration in critically ill patients with sepsis-associated AKI. The rationale behind the renal protective effects remains to be fully elucidated, but is likely to be related to dephosphorylation and thereby detoxification of detrimental molecules involved in the pathogenesis of sepsis-associated AKI. A potent candidate target molecule might be endotoxin (lipopolysaccharide) from the cell wall of Gram-negative bacteria, which is associated with the development of sepsis and becomes nontoxic after being dephosphorylated by alkaline phosphatase. Another target of alkaline phosphatase could be adenosine triphosphate, a proinflammatory mediator released during cellular stress, which can be converted by alkaline phosphatase into the tissue-protective and anti-inflammatory molecule adenosine. Human recombinant alkaline phosphatase, a recently developed replacement for bovine-derived alkaline phosphatase, has shown promising results in the preclinical phase. As its safety and tolerability were recently confirmed in a phase I clinical trial, the renal protective effect of human recombinant alkaline phosphatase in sepsis-associated AKI shall be investigated in a multicenter phase II clinical trial starting at the end of this year.

Introduction

Sepsis represents an important syndrome in the intensive care unit (ICU). This infectious-inflammatory disease, which can ultimately result in loss of organ function and death, has an incidence of 3 cases per 1,000 individuals. In addition, more than 50% of the patients are admitted to the ICU, with a mortality of approximately 1 out of

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3 patients [1]. A common consequence of sepsis is the development of acute kidney injury (AKI). AKI is characterized by a rapid loss of kidney function resulting in an attributable mortality; 2 out of 3 patients suffering from sepsis-associated AKI die [2]. Patients surviving an episode of sepsis-associated AKI have an increased risk of developing end-stage renal disease, resulting in a tremendous burden for both the patient and society [3]. Sepsis-associated AKI is believed to be a multifactorial disease caused by the interplay of an inflammatory insult, altered microcirculation, and unbalanced renal bioenergetics [4]. Unfortunately, a pharmacological treatment for AKI is currently unavailable, most likely due to the complex pathogenesis of this disease. Since only supportive care (e.g. renal replacement therapy) can be offered to these patients, the medical need for a new pharmacological treatment option is urgent. In the past, several attempts to find such a therapy have been made, but all promising candidates have failed in clinical phases [5, 6]. Recently, two small phase II trials suggested that the administration of bovine alkaline phosphatase (AP), an endogenous dephosphorylating enzyme, might exert beneficial effects on kidney function in patients suffering from sepsis [7, 8]. Here, we elaborate on the latest clinical data, the postulated mechanism of action, and the future of AP as a highly potent treatment option for critically ill patients with sepsis-associated AKI.

Alkaline Phosphatase

AP is a membrane-bound enzyme which can dephosphorylate, and thereby detoxify, harmful molecules. AP is expressed throughout the entire body and originates from 4 different isoenzymes: germ cell AP, intestinal AP, placental AP, and tissue nonspecific (liver/bone/kidney) AP [9]. Originally, AP was developed as an antisepsis drug, as it can dephosphorylate lipopolysaccharide (LPS). This endotoxin, part of the outer membrane of Gram-negative bacteria, is a pathogen-associated molecular pattern involved in the development of sepsis. LPS binds, together with the LPS-binding protein, to Toll-like receptor 4 (TLR4), to which the cell-surface molecule MD-2 and the coreceptor CD14 are attached. The activation of TLR4 by LPS triggers intracellular MyD88-dependent and MyD88-independent pathways, followed by activation of the transcriptional factor nuclear factor-κB. Eventually, this results in the production of cytokines and other effector molecules, thereby enhancing the inflammatory response [10]. AP is able to detoxify LPS by removing 1 of the 2 phosphate groups of the lipid A part of LPS, which normally causes its toxicity [11]. This results in a nontoxic LPS molecule that can still bind to TLR4 but now acts as an antagonist, preventing activation of the inflammatory cascade.

After confirming the safety and clinical pharmacology of bovine intestinal AP (biAP) both in healthy volunteers and in severe sepsis patients [12], biAP was studied in a phase IIa clinical trial with severe sepsis or septic shock patients, with or without AKI, admitted to the ICU [7]. Surprisingly, while biAP did not influence the severity or course of sepsis, it did show renal protective effects. Compared to placebo, biAP treatment resulted in lower median plasma creatinine levels, reduced urinary excretion of the proximal tubule injury marker glutathione-S-transferase A1, and attenuation of the upregulation of inducible nitric oxide synthase in proximal tubule cells isolated from urine, causing an attenuated excretion of urinary NO metabolites. Also, biAP appeared to prevent the development of AKI in sepsis patients who did not suffer from AKI when included in the study, and it was suggested to reduce the need for renal replacement therapy in the 28-day follow-up period in sepsis patients with AKI. While the number of patients was too small to reach statistical significance for the latter clinical parameters, these results were encouraging and formed the basis of a second prospective phase IIa clinical trial with biAP in which critically ill patients were included with severe sepsis or septic shock with evidence of early AKI [8]. Interestingly, biAP treatment improved the endogenous creatinine clearance in these patients and reduced the need for and duration of renal replacement therapy compared to placebo, confirming a renal protective effect. Moreover, markers of systemic inflammation (LPS-binding protein, interleukin-6, and C-reactive protein) returned to baseline more swiftly and the urinary excretion of renal injury markers kidney injury molecule-1 and interleukin-18 was lower in the biAP-treated group compared to the placebo-treated group. Although both studies were carried out in a relatively small number of patients, the results indicated renal protective effects of AP in critically ill patients, underlining the need for further research on AP as a new biological agent to treat sepsis-associated AKI.

Mechanism of Action

The beneficial renal protective effects of AP have already been demonstrated in the critically ill; however, the precise mechanism of action of AP remains to be eluci-

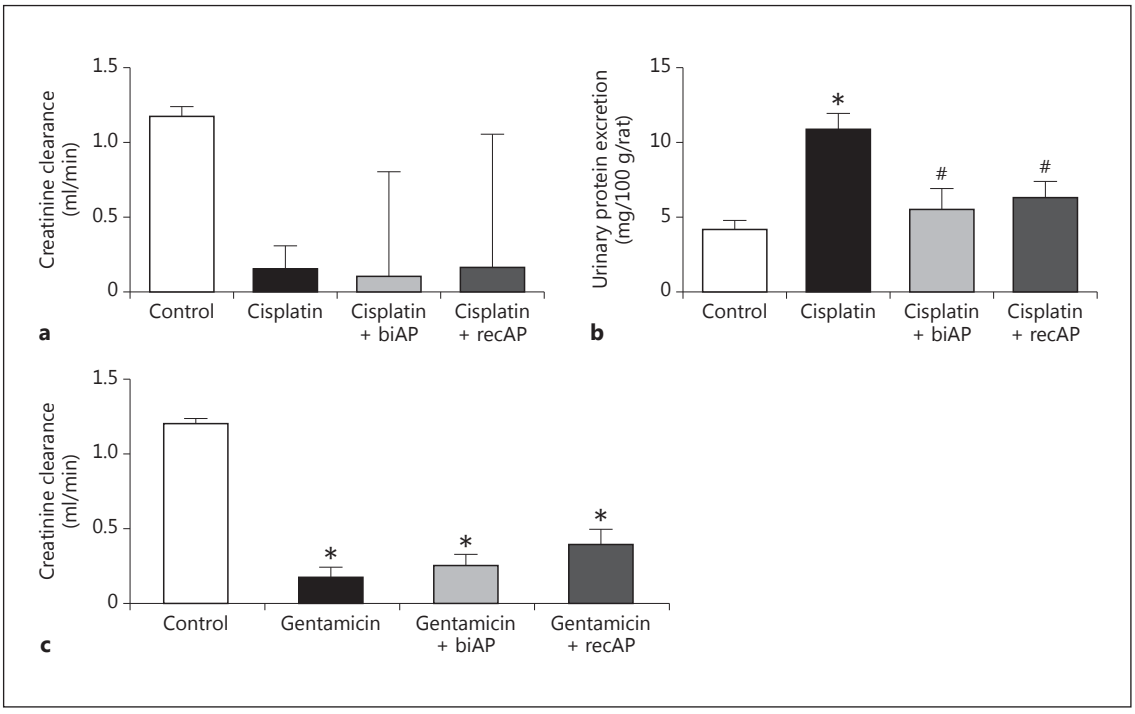

Fig. 1. Effects of biAP and human recAP on renal function in cisplatin- and gentamicin-induced AKI in Wistar rats. **a**, **b** AKI was induced by a single intraperitoneal injection of 7.5 mg/kg cisplatin on day 0, causing reversible renal impairment in rats as manifested by, for example, elevated serum creatinine levels and reduced creatinine clearance, with injury peaking on day 3. As treatment, rats (n = 8 per group) received biAP or recAP intravenously at 200 U/kg twice a day on days 0 and 1 and an additional dose on day 2. The first treatment dose was given 30 min before the cisplatin challenge. Creatinine clearance (**a**) and urinary protein excretion (**b**) were determined on day 3. **c** Rats were administered gentamicin (120 mg/kg) intramuscularly for 7 consecutive days to induce AKI, which manifested as, for example, increased serum creatinine levels and reduced creatinine clearance. Rats (n = 8 per group) received twice-daily intravenous injections of 100 U/kg AP (biAP or recAP) over 7 days, with the first dose given immediately prior to the daily gentamicin challenge. Creatinine clearance was determined on day 7. Data are presented as medians (IQR) (**a**) or means ± SEM (**b**, **c**). Significant differences were estimated using a one-way ANOVA with Bonferroni's posttest, or a Kruskal-Wallis test with Dunn's posttest. * $p < 0.05$ compared to placebo; # $p < 0.05$ compared to cisplatin.

dated. Considering the dephosphorylating properties of AP, target candidates might be detrimental molecules such as LPS or adenosine triphosphate (ATP), both of which lose their proinflammatory properties after being dephosphorylated. The protective effects of AP on LPS-induced inflammation have been demonstrated in several in vivo models. Survival rates improved significantly in mice treated with AP compared to placebo after being exposed to a sublethal dose of LPS [11, 13]. Also, AP treatment resulted in reduced serum NO levels and attenuated plasma cytokine peak levels, fever, and organ damage during systemic inflammation [13–15]. Oral administration of an endogenous AP inhibitor increased serum LPS levels, suggesting that the beneficial effects of AP could be ascribed to a decrease in serum LPS content [16]. Furthermore, in endothelial cells the LPS-induced activity of nuclear factor-κB was reduced following AP treatment, signifying an effect of AP on cellular LPS-TLR4 signaling [17]. All of these effects of AP treatment are suggested to be due to dephosphorylation and thereby detoxification of LPS, which was demonstrated by the AP-mediated presence of free phosphate derived from LPS in both in vitro and in vivo studies [11, 14].

Another detrimental molecule involved in the pathogenesis of sepsis is extracellular ATP. Under physiological conditions, this energy molecule resides within the cell but, during cellular stress induced by, for example, inflammation or hypoxia, ATP is released. Extracellular ATP then acts as a proinflammatory mediator and enhances further inflammation and tissue injury [18]. As part of our body's own defense mechanism, ATP can be dephosphorylated by ectonucleotidases into adenosine

diphosphate, adenosine monophosphate, and ultimately adenosine. Adenosine, normally acting as a regulator of, for example, the glomerular filtration rate and tubular glomerular feedback, can exert anti-inflammatory and tissue protective effects by binding to 1 of the 4 adenosine receptors, i.e. A1, A2A, A2B, or A3 [19]. The beneficial effects of adenosine on both systemic inflammation and renal injury have been demonstrated in several animal studies. Treatment of an adenosine receptor A2A agonist or a synthetic adenosine analogue improved survival rates in mice exposed to LPS [20, 21], and adenosine receptor A1 and A3 deficiency resulted in increased renal dysfunction and mortality rates [22, 23]. Considering the anti-inflammatory and tissue-protective effects of adenosine and the proinflammatory effects of extracellular ATP during cell stress, enhancement of the conversion of ATP into the protective molecule adenosine might be beneficial. Interestingly, AP is able to completely hydrolyze ATP into adenosine, indicating that exogenous AP might be an elegant method to restore the nucleotide balance, which could explain the renal protective effect during sepsis-associated AKI.

Future Perspectives and Conclusion

Considering the potential of AP as a new treatment for sepsis-associated AKI, human recombinant AP (recAP) has currently been developed to replace the bovine-derived intestinal AP that was previously used in phase II clinical trials. This chimeric construct consists of the crown domain of placental AP, which is incorporated into the intestinal AP structure to improve enzyme stability, while maintaining its catalytic function [24]. Recently published in vitro data confirmed the dephosphorylating capacity of recAP towards LPS and ATP [24]. Our preliminary in vitro results demonstrated that recAP can attenuate LPS-induced cytokine production in a human renal cell line [25], whereas recAP improves renal blood flow and vascular resistance and inhibits various parameters of renal inflammation and tissue damage during AKI induced by ischemia-reperfusion or by LPS injection in vivo [26]. Interestingly, recAP tends to also exert renal protective effects during cisplatin- or gentamicin-induced AKI, which may indicate a broader use for recAP treatment than sepsis-associated AKI alone (fig. 1).

As the safety and tolerability of recAP were recently confirmed in a phase I clinical trial with healthy volunteers, the potency of recAP as a treatment for sepsis-associated AKI in critically ill patients shall be studied in a multicenter phase II clinical trial, with endogenous creatinine clearance as the primary endpoint [27].

In conclusion, AP is suggested to be a potent new treatment option for sepsis-associated AKI, as demonstrated by preclinical data and two phase II clinical trials, and it might exert its renal protective effect through detoxification of LPS and/or ATP. The upcoming phase II clinical trial will unveil the true significance of AP treatment in order to prevent or cure the development of sepsis-associated AKI.

Disclosure Statement

P. Pickkers declares having received speaking and consultation fees from AM-Pharma (the manufacturer of AP). E. Peters and R. Masereeuw have no financial conflicts of interest to declare.

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