Nephrol Dial Transplant, 2024, **39**, 1239–1247
https://doi.org/10.1093/ndt/gfae028
Advance access publication date: 23 February 2024

REVIEW

Alkaline phosphatase treatment of acute kidney injury—an update

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ABSTRACT

Through improved insights into the increasing incidence and detrimental effects of acute kidney injury (AKI), its clinical relevance has become more and more apparent. Although treatment strategies for AKI have also somewhat improved, an adequate remedy still does not exist. Finding one is complicated by a multifactorial pathophysiology and by heterogeneity in the patient population. Alkaline phosphatase (ALP) has been suggested as a therapy for sepsis-associated AKI because of its protective effects against lipopolysaccharide (LPS)-induced inflammation and kidney injury in animals. However, its effectiveness as an AKI treatment has not been demonstrated definitively. Because the anti-inflammatory properties of ALP are likely not reliant on a direct effect on LPS itself, we postulate that other pathways are much more important in explaining the renoprotective properties ascribed to ALP. The re-evaluation of which properties of the ALP enzyme are responsible for the benefit seen in the lab is an important step in determining where the true potential of ALP as a treatment strategy for AKI in the clinic lies. In this review we will discuss how ALP can prevent activation of harmful pro-inflammatory receptors, redirect cell–cell signalling and protect barrier tissues, which together form the basis for current knowledge of the role of ALP in the kidney. With this knowledge in mind and by analysing currently available clinical evidence, we propose directions for new research that can determine whether ALP as a treatment strategy for AKI has a future in the clinical field.

Keywords: AKI, alkaline phosphatase, barrier function, ischaemia–reperfusion injury, purinergic signalling

INTRODUCTION

Acute kidney injury (AKI), defined by an acute increase in serum creatinine levels or a decrease of urine production, affects 20% of all hospitalized patients and is associated with clinically significant mortality, ranging from 20 to 36% in the critically ill [1]. Despite growing insights into the impact of AKI and in its pathophysiology, effective AKI treatment is lacking.

AKI is triggered by disturbances of the body's homeostasis—as can frequently be observed in patients with sepsis, trauma and heart failure—and iatrogenic interventions including surgery and medication administration or discontinuation [2]. These disturbances result in kidney damage either directly (e.g. through toxic effects) or through inflammation and ischaemia–reperfusion injury (IRI) [3]. The latter phenomenon starts with hypoxic damage, which is then aggravated by oxygen radicals that are released during the reperfusion phase. IRI forms the basis of AKI pathophysiology in cardiothoracic surgery and kidney transplantation, however, it is also often present in sepsis-associated AKI (SA-AKI) [1].

Current AKI treatments, e.g. haemodynamic support and antibiotic treatment, are primarily focused on the cause of the homeostatic disturbance and can themselves also aggravate kidney damage [3]. Excessive fluid administration can lead to renal congestion and microcirculatory impairment [4]. Antibiotics such as aminoglycosides are known for their nephrotoxic effects, which are observed in 10–20% of aminoglycoside treatments and can lead to tubular cell death directly [5].

Whether or not an outside trigger leads to AKI is determined by the extent of the direct damage, individual susceptibility to kidney damage and the host-response that is generated to the insult [1]. Improved insights into host-response profiles associated with AKI has identified potential targets for AKI treatment.

In sepsis and renal ischaemia, the expression of biomarkers in the inflammatory, coagulation and endothelial damage domains are correlated with AKI severity [6, 7]. Also, in patients with severe COVID-19, stronger anomalies in host-response profiles are associated with AKI persistence [8].

It has been hypothesized that supplementation of alkaline phosphatase (ALP) can alter certain aspects of the host-response to pathogens and injury. To understand how this could be used to treat AKI, we must understand the physiologic functions of ALP, its interaction with the host-response and the role of the host-response in AKI pathophysiology. These basic subjects will be discussed together with evidence from clinical trials.

Received: November 2, 2023; Editorial decision: January 22, 2024

ALP

ALP is an endogenous enzyme that is expressed in bone and physiological barrier tissues such as the intestinal tract and kidney tubules. There are at least four iso-enzymes of ALP, which are named after their primary site of expression. The main function of all ALP iso-enzymes is the dephosphorylation (removal of phosphate) of other proteins, which alters the function of these proteins [9].

The differences between the different iso-enzymes are demonstrated by their degree of enzymatic activity, chemical properties and primary site of expression. Their evolutionary preservation suggests a unique role for each of them [10]. For instance, placental ALP (PLALP) is the least efficient iso-enzyme, with a catalytic constant that is less than one-fifth of that of intestinal ALP (IALP, discussed later) [10]. PLALP is very heat resistant; more so than all the other iso-enzymes [9]. It is also known to play a role in the transfer of immunoglobulin G molecules from the mother to the foetus during gestation [10]. In contrast, little is known about germ-cell ALP's specific functions and it is only expressed on embryonal cells and in some neoplastic tissues.

Tissue non-specific ALP (TNALP) is expressed in bone, liver and kidney tissue and makes up practically all circulating ALP. Normal TNALP function is essential for bone mineralization, however, it also contributes to vascular calcification and possibly to the genesis of Alzheimer's disease [9]. Administration of exogenous TNALP has been approved for the treatment of bone demineralization in severe hypophosphatasia.

IALP plays a role in maintaining gut homeostasis by regulating gut microbiome tolerance and lipid absorption [11]. Because of extensive evidence for its role in the immune response to pathogens in the gut and its superior enzymatic activity, it has been proposed that IALP can be used as a treatment for inflammatory disorders [e.g. SARS-CoV-2 infection (EUdraCT: 2020-001714-38), ulcerative colitis [12] and AKI [13]]. It is available as a bovine-sourced preparation (bIALP) and in a human recombinant form (recALP). The latter resembles IALP's biologic activity and PLALP's superior heat stability [14]. For more accessible reading, the endogenous and the medicalized isoforms of ALP will be referred to as 'ALP' throughout the rest of this review.

The host-response

The kidney injury–inducing host-response is mediated by signalling molecules, from which two families can be distinguished based on what type of cell has released them. These families are known as pathogen- and damage-associated molecular patterns (PAMPs and DAMPs, respectively) [15]. PAMPs are exogenous or endogenous microorganism-derived molecules, such as lipopolysaccharides (LPS; a toxic constituent of Gram-negative bacteria). External PAMPs can enter the body through a primary infectious site, whereas endogenous PAMPs enter through the intestinal barrier. The latter phenomenon is known as gut translocation, which results from intestinal ischaemia. Intestinal ischaemia can arise due to diseases such as sepsis or due to surgery and vasopressive therapy [16]. DAMPs are molecules produced by the body itself and are either actively released in response to distress or passively leak from dead or damaged cells [17].

PAMPs and DAMPs induce kidney damage by activation of toll-like receptors (TLRs) and purinergic or pyrimidinergic receptors. In rodent models of sepsis and kidney IRI, inhibition of either TLR4 [18] or the purinergic P2X7 receptor protects against AKI [19, 20].

PAMPs and AKI: ALP in the TLR pathway

TLRs are transmembrane glycoproteins that can recognize both PAMPs and DAMPs and induce both the innate and adaptive immune system (Fig. 1). There are 10 subtypes of TLRs in humans, of which TLR4 has been studied most extensively with respect to AKI pathophysiology [21]. ALP does not inhibit TLRs directly, but it has been hypothesized that ALP can prevent activation by the dephosphorylation of TLR ligands.

The first mechanism of action that was proposed to explain the anti-inflammatory properties of ALP was the direct detoxification of TLR4's main substrate: LPS. The bulk of ALP-related research has been built around this proposed mechanism, which sparked special interest in its use as a treatment for Gram-negative sepsis and endotoxemia [11, 22].

The potency of LPS as a TLR4 agonist is determined by a structure inside the LPS molecule called the lipid A subunit. This lipid A subunit contains two phosphate groups that are vital to its activation but not its binding to TLR4 [23]. Therefore, once the lipid A subunit is dephosphorylated, LPS works as a TLR antagonist.

Peters *et al.* [23] found that LPS-induced kidney damage, inflammatory biomarker expression and glomerular filtration rate reduction were alleviated by ALP co-administration in rats. These findings were strengthened by histologic findings of reduced cell damage and swelling in the ALP-treated group. Together with earlier studies demonstrating that supplemented ALP can release inorganic phosphate (Pi) when co-incubated with LPS, it was generally assumed it also dephosphorylated lipid A [24].

However, Komazin *et al.* [25] demonstrated that ALP has only very limited substrate specificity for LPS and found no evidence for dephosphorylation of the toxic TLR4-stimulating lipid A subunit by ALP. Rather, the released phosphate observed in earlier studies probably originates from a different part of the molecule. The effects asserted by ALP on LPS-induced inflammation are likely mediated through different mechanisms than direct detoxification.

Other PAMPs have also been proposed as substrates for ALP. In 2010, Chen *et al.* [26] exposed endothelial cells to bacteria-derived flagellin and CpG DNA motifs. After co-incubation with ALP, the release of pro-inflammatory cytokine interleukin 8 (IL-8) was reduced and the concentration of free phosphate increased.

Flagellin induces inflammation and oxidative stress through TLR5, which can work in synergy with TLR4 to create a more potent immune response. The role of TLR5 in AKI is ambiguous, as was demonstrated by Fukuzawa *et al.* [27], who showed—with a murine model of renal IRI—that a TLR5 agonist can protect against AKI at low-dose administration but not at high-dose administration.

CpG DNA motifs stimulate TLR9, which is—unlike TLR4 and TLR5—located inside the cell. Tsuji *et al.* [28] found that TLR9 knockout (KO) mice are protected from AKI during sepsis, which suggests a prominent harmful role for TLR9 in AKI pathophysiology. This is especially notable because TLR4 KO is not associated with protection against AKI [29].

ALP can release Pi from at least three PAMPs that have been associated with AKI pathogenesis and can reduce the inflammatory response to these PAMPs. However, the release of Pi from a PAMP does not necessarily limit its toxicity. For LPS, it is likely that toxicity is not significantly affected; for flagellin and CpG motifs, this is yet to be determined. In Table 1, an overview of (potential) substrates for ALP, their respective products and relevant effects for AKI pathophysiology are presented.

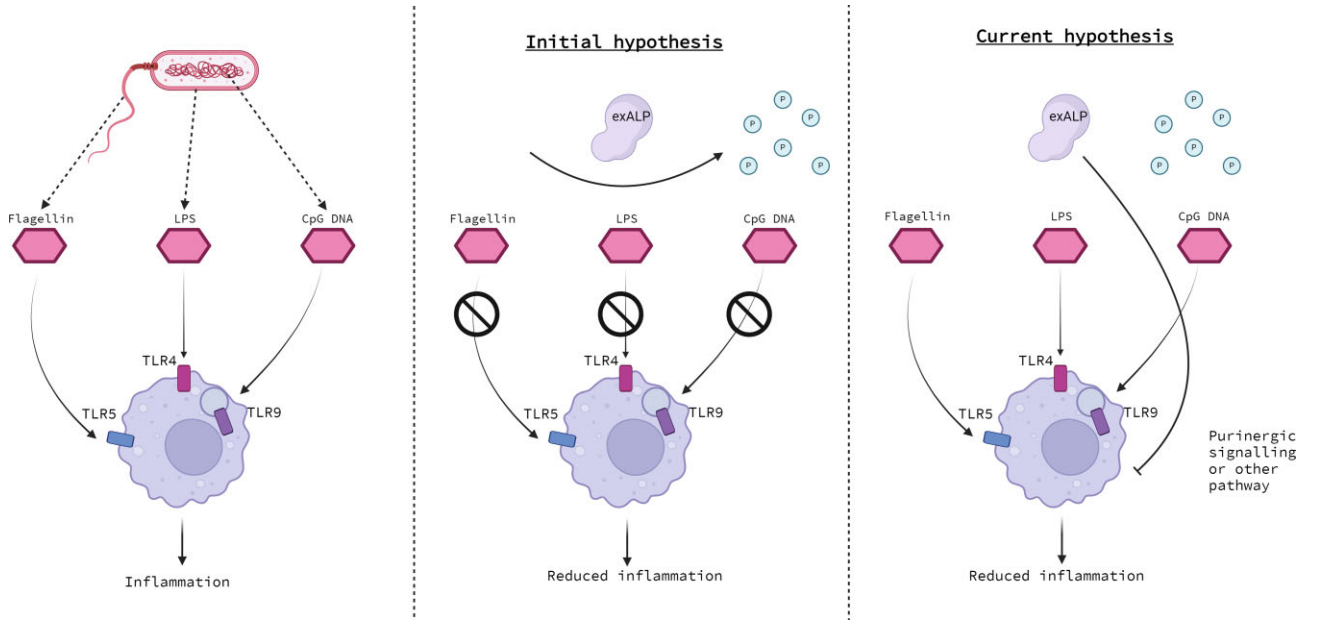

Figure 1: Initial and new hypotheses for interaction of ALP with PAMPs. In this figure, a generic inflammatory cell is depicted. PAMPs released from Gram-negative bacteria cause a potent inflammatory response through TLRs. Despite current evidence pointing away from direct detoxification of the toxic lipid A core in LPS by ALP, treatment with ALP leads to strongly reduced PAMP-induced inflammation *in vivo* and *in vitro*. These effects might be mediated through purinergic/pyrimidinergic signalling or through a yet undetermined pathway. For flagellin and CpG DNA, indirect evidence suggests reduced toxicity, which needs to be confirmed with additional testing. Arrows indicate activation; arrow with flat head indicates inhibition. Created with BioRender.com.

Regardless of a direct effect of ALP on PAMP toxicity, the anti-inflammatory effects observed *in vitro* and *in vivo* are evident. Another pathway in the dampening effect of ALP on the host-response to injury might be involved.

DAMPs and AKI: the role of ALP in the purinergic signalling pathway

In response to sterile and infectious stimuli (e.g. bacteria or PAMPs), epithelial and endothelial cells, immune cells and platelets release signalling molecules in the form of extracellular nucleotides and nucleosides (DAMPs). A nucleotide is a phosphorylated nucleoside containing up to three phosphate groups, and nucleosides derive from the building blocks of DNA. They are released from cells either passively (due to cell damage), through active transport molecules like pannexin and connexin or are formed from other extracellular nucleotides [17].

ALP can generate nucleosides from nucleotides by dephosphorylation, and these nucleosides exert completely different effects on receptor cells than their nucleotide counterparts. The nucleosides adenosine and uridine form the basis for purinergic and pyrimidinergic signalling, respectively [30]. Their nucleotide forms are adenosine monophosphate (AMP), adenosine diphosphate (ADP) and adenosine triphosphate (ATP) and uridine diphosphate (UDP) and uridine triphosphate (UTP). In the following paragraphs we will discuss the role purinergic and pyrimidinergic signalling plays in AKI. A synoptic overview of this is provided in Fig. 2.

Purines

At physiologic levels, extracellular ATP stimulates P2X and P2Y receptors, which are vital to normal T cell function. At high levels, it activates the P2X7 receptor, which causes tissue damage by pro-inflammatory macrophage polarization and activation of pro-inflammatory T-helper cells [17]. Extracellular ADP acts on P2Y receptors, which promote the release of pro-inflammatory cytokines and cause platelet aggregation [17, 31].

Extracellular adenosine has mostly opposite effects from ATP and ADP through activation of the P1 receptor, which has four subtypes. Activation of the A1 and A3 subtypes leads to decreased intracellular cyclic AMP (cAMP) levels and activation of the A2 subtypes leads to increased intracellular cAMP levels. Furthermore, A1, A2a and A3 receptors have a high affinity for adenosine, whereas the A2b receptor has a much lower affinity for adenosine. The main takeaway should be that the effects of extracellular adenosine can vary and even seem to oppose each other based on the extent of stimulation of a certain receptor (Fig. 2). Monaghan *et al.* [32] have provided a comprehensive review of the role of P1 and P2 receptors in kidney health and disease.

Adenosine has an inhibitory effect on platelets, reduces inflammation, induces repair of endothelial cells and protects against extravasation of fluids during ischaemia [17]. It also activates the tubuloglomerular feedback system, which causes vasoconstriction of the afferent arteriole by which renal medullar oxygen consumption is decreased [30]. During IRI in kidney transplantation, activation of regulatory T cells by adenosine potentially results in reduced acute and chronic rejection [33].

The conversion of extracellular ATP and ADP into adenosine occurs in three steps by the endothelial- and B cell–bound ectonucleotidases CD39 and CD73, or in one step by endogenous ALP [17]. *In vitro* experiments show that exogenous ALP can also remove all phosphate groups from ATP and ADP to produce adenosine [23].

In 2022, Rosin *et al.* [34] showed that pretreatment with ALP offered protection against AKI in mice and rats exposed to renal artery clamping (26 and 30 min) and subsequent reperfusion. Treatment with an A2a receptor antagonist completely nullified this effect (mice), which suggests a prominent role for

Table 1: List of (possible) substrates for ALP as mentioned in this article.

Substrate/molecule of interest	Product	*In vitro* results of ALP co-incubation	*In vivo* results	Altered by ALP treatment	References
LPS (PEtN)	LPS	Inhibited pro-inflammatory cytokine induction	Reduced damage in LPS-induced kidney injury	Unlikely	Komazin *et al.* [25], Peters *et al.* [23]
Flagellin	Unknown	Pi generation Inhibited pro-inflammatory cytokine induction	Direct evidence unavailable	Possible	Chen *et al.* [26]
CpG DNA	Unknown	Pi generation Inhibited pro-inflammatory cytokine production	Direct evidence unavailable	Possible	Chen *et al.* [26]
ATP/ADP	Adenosine	Inhibited pro-inflammatory cytokine production Inhibited sepsis-induced platelet aggravation	Reduced damage in IRI-induced kidney injury through adenosine production	Certain	Tunjungputri *et al.* [31], Rosin *et al.* [34]
UTP/UDP	Uridine	Inhibited pro-inflammatory cytokine production	Reduced UDP-induced inflammation	Certain	Moss *et al.* [37]
TJPs	Altered phosphorylation State of TJPs	ALP can dephosphorylate TJPs	Increased TJ expression Improved TJ localization	Possible (indirect)	Harmaneh *et al.* [42], Liu *et al.* [47], Plaeke *et al.* [56], Sakakibara *et al.* [48]
Live bacteria	Unknown	Cell-bound ALP limits inflammation Exogenous ALP does not limit inflammation	Direct evidence unavailable	Unknown	Chen *et al.* [26]

List of relevant molecules mentioned in literature as possible substrates for ALP. Komazin *et al.* [25] found that the true substrate for ALP in the co-incubation with LPS is probably spontaneously hydrolysing PEtN groups rather than the lipid A subunit as had previously been hypothesized. Although TJPs can be dephosphorylated by ALP, it is likely that this does not happen in living cells. Rather, an indirect pathway might be responsible for any alterations.

extracellular adenosine [34]. Interestingly, A2a receptor antagonism does not impair the protective effects of ALP against LPS-induced associated AKI [23], which suggests that ALP renoprotection is not dependent on a single mechanism of action or on a single adenosine receptor. The latter is supported by that A2a receptor KO reduces but does not nullify the protective effects of ALP against renal IRI [34].

ALP administration also reduces platelet activation by reducing extracellular levels of ADP and increasing adenosine levels. Tunjungputri *et al.* [31] demonstrated in an *ex vivo* setting that ALP can reverse sepsis-induced hyperactivity of human platelets. Platelets are an important part of the host-response and hyperactive platelets cause inflammation and endothelial damage. A reduction of ADP-induced platelet activation—through inhibition of the P2Y12 receptor with clopidogrel—has protective effects during renal IRI in mice [35].

Pyrimidines

The pyrimidinergic nucleotides UTP and UDP exert pro-inflammatory effects, mainly through P2Y receptors. Although no specific receptor has yet been identified for uridine, in a study of murine lung injury, exogenous uridine supplementation attenuated inflammation and fibrosis [36].

A 2013 *in vitro* study by Moss *et al.* [37] showed that ALP can inhibit the inflammatory response elicited by UDP on monocytes. In this study, ALP did not limit IL-8 production after LPS stimulation unless co-incubated with UDP. This is suggestive of a role for the pyrimidinergic pathway in the earlier described studies that showed protective effects of ALP in LPS-induced AKI. The viability of uridine as an immunosuppressive molecule is further supported by the finding that ALP treatment alleviated inflammation after tumour necrosis factor-α (TNF-α) stimulation when co-incubated with UDP [37]. TNF-α is not a substrate for ALP, however, TNF-α does activate connexin and pannexin in some cells, including venous endothelial cells [38]. Dephosphorylation of the consequently released nucleotides by ALP could reduce TNF-α-induced monocyte extravasation and inflammation.

A study by Peters *et al.* [22] described protective effects of ALP treatment against gentamicin- and cisplatin-induced AKI. Although the authors did not test hypotheses for a protective mechanism of action, purinergic signalling could also be involved here. Increased oxidative stress is a vital part of medication-induced AKI and a downregulator of CD39 and CD73 [5, 39]. The increased purinergic signalling through P2 receptors (ATP, ADP, UTP, UDP), which is consequential to this downregulation of CD39 and CD73, aggravates the oxidative stress–induced damage [39]. ALP can compensate for the lost capacity of purine and pyrimidine nucleotide conversion. Adenosine receptor stimulation alone was demonstrated to be protective against cisplatin-induced AKI in rats by Gill *et al.* in 2009 [40].

To summarize: purinergic and pyrimidinergic receptor substrates can be directly altered by ALP, which can break the vicious cycle of damage by interaction of activated endothelium, inflammation and coagulation. Additionally, despite the apparent inability of ALP to detoxify lipid A in LPS, there is another way by which ALP can prevent the harmful effects of TLR activation.

The barrier enzyme: ALP and tight junctions in AKI

ALP is essential to the structural integrity of border tissues (such as endothelium and the tubular epithelium) because of its close

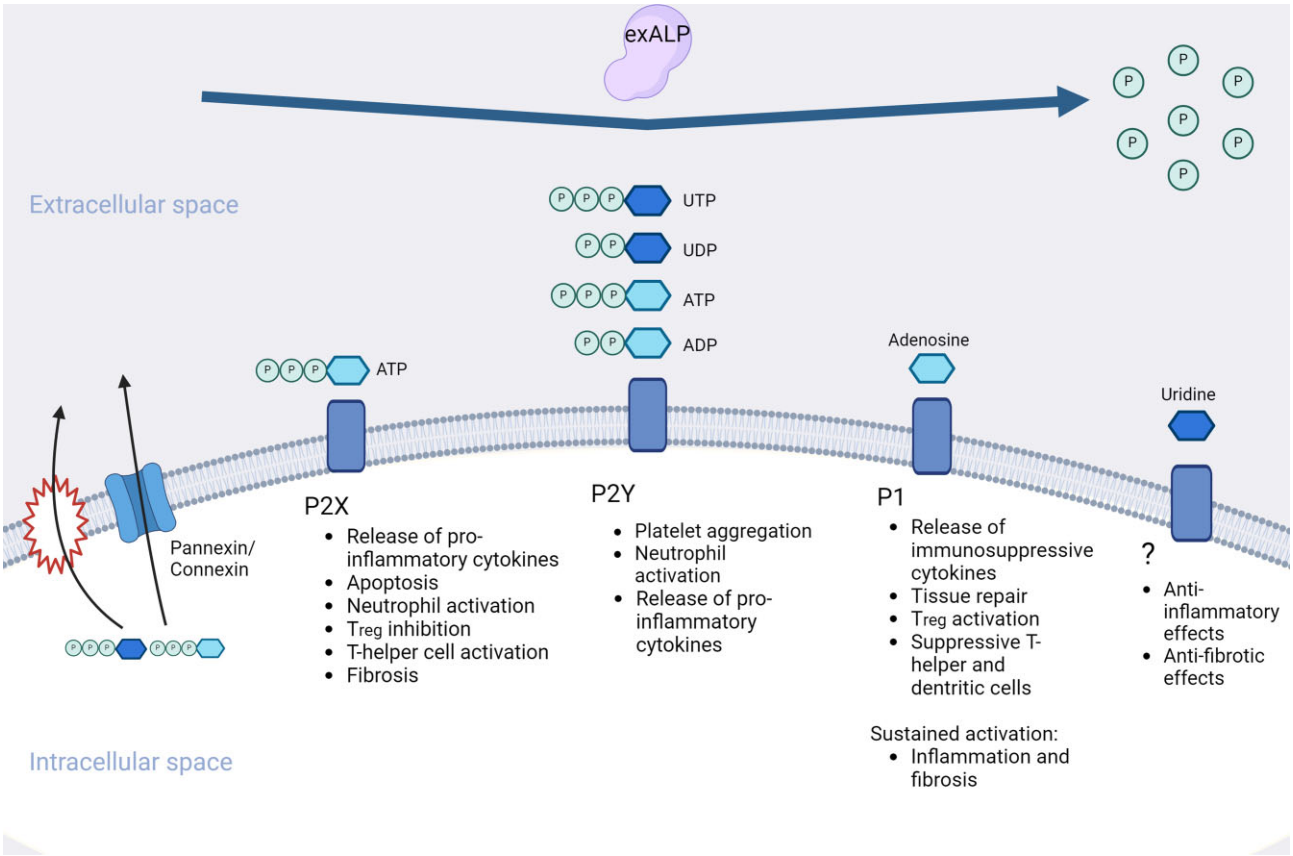

Figure 2: Purinergic receptor pathway. Intracellular nucleotides (ATP and UTP) are released from damaged (active through connexin or pannexin) or necrotic cells (passive leakage). In the extracellular space, these nucleotides are potent activators of pro-inflammatory receptors. They can be dephosphorylated by membrane proteins CD39 and CD73 (not shown) one phosphate group at a time and eventually into their base molecule (adenosine or uridine). ALP is the only enzyme known to remove all phosphate groups from ATP and UTP at once, directly producing adenosine and uridine. ADP and UDP also activate pro-inflammatory signalling receptors. Adenosine activates the P1 receptors and thereby acutely reduces inflammatory damage. Long-term activation of adenosine receptors leads to inflammation and fibrosis. Anti-inflammatory effects are also attributed to uridine; however, it is unknown through which pathway these effects are induced. Exogenous ALP (exALP) releases phosphate from nucleotides to form adenosine and uridine. Arrow indicates dephosphorylating action. Created with BioRender.com.

relationship with tight junctions (TJs). TJ proteins (TJPs) are vital to both endothelial and epithelial barrier function and the preservation of TJP function can protect kidney function against IRI *in vivo*. The role of TJPs in AKI pathophysiology and the therapeutic potential of targeting them have recently been reviewed by Wei *et al.* [41].

Harmaneh *et al.* [42] demonstrated in 2014 that orally administered exogenous ALP increased TJP mRNA expression and reversed starvation-induced epithelial barrier dysfunction. Thus ALP can prevent leakage of endotoxins into the circulation and prevent them from activating TLRs. The importance of this remote activation was demonstrated by Liu *et al.* [43], who showed a TLR4-dependent protective effect of oral ALP administration on liver fibrosis.

As well as reducing TLR activation, the protection of barrier tissues might also protect against tubular damage directly. In the kidney, ALP is expressed in both the proximal and distal tubules [44]. Presbitero *et al.* [45] noted that during cardiothoracic surgery with cardiopulmonary bypass in humans, a surge of endogenously released ALP can be observed—depleting stores of tissue ALP. Supplemented exogenous ALP protects the activity of ALP that is already present in the body [46]. An interesting note here is that Chen *et al.* [26], whose work was described earlier in this review, also demonstrated that cell-bound ALP limits inflammation induced by live bacteria, but exogenous ALP does not. This stresses the importance of endogenous ALP protection by ALP supplementation.

In 2019, Davidson *et al.* [46] demonstrated in a piglet model of cardiopulmonary bypass with circulatory arrest that infusion of exogenous ALP protected kidney tissue ALP activity and was associated with a lower incidence of AKI. Thus supplementation with exogenous ALP preserves tissue ALP and protects brush border barrier function by preservation of TJP expression and localization (Fig. 3) [12, 46, 47].

Although ALP can dephosphorylate TJPs, the prominent phosphorylation sites of TJPs are located intracellularly and are thus probably inaccessible to exogenous and cell-bound ALP [48, 49]. Because purines are known to influence TJP expression, an indirect pathway through which ALP regulates TJP expression and localization might be more likely [50]. To summarize: treatment with exogenous ALP can alleviate endothelial and epithelial damage, vascular leakage, inflammation, oxygen consumption and platelet activation through the conversion of DAMPs and can protect endogenous ALP activity, which subsequently preserves barrier function in the gut and possibly in the kidney. Whether these effects are strong enough to treat or protect against AKI will have to be demonstrated by clinical trials.

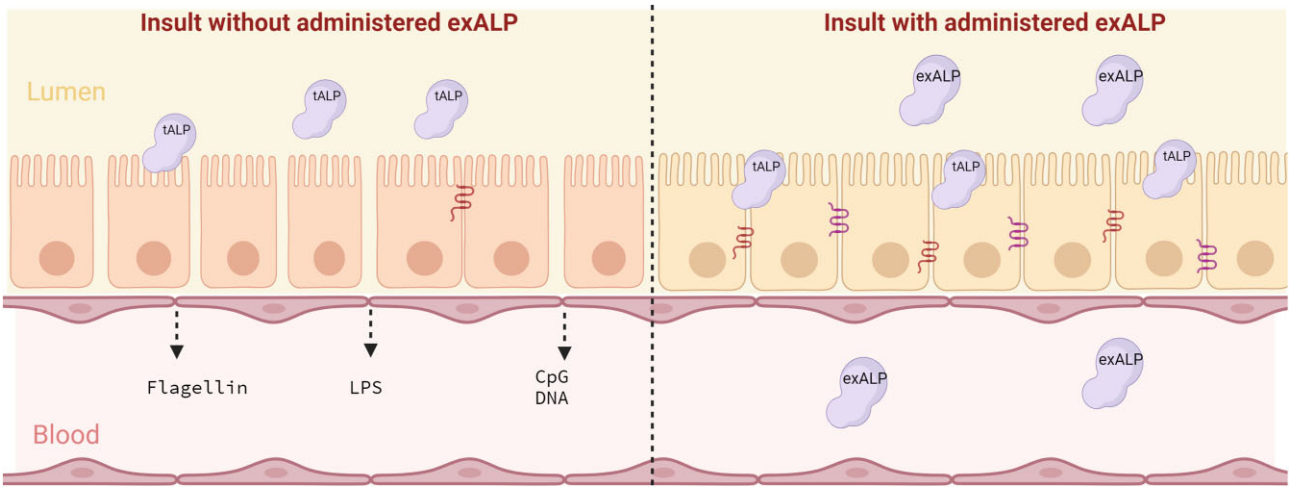

Figure 3: Loss of tissue ALP (tALP) is associated with a loss of intestinal barrier function and increased leakage of endotoxins. Oral and parenteral supplementation with exogenous ALP (exALP) preserves tALP activity and TJPs during (ischaemic) insults and protects intestinal epithelial barrier function. tALP offers protection against bacteria-induced inflammation whereas exALP does not. There are similarities between ALP expression in the intestinal and tubular epithelium, however, its specific functions in the latter have yet to be elucidated. Created with BioRender.com.

Table 2: Placebo-controlled randomized clinical trials with exogenous ALP.

Clinical trial	Setting	Groups	Kidney-related outcomes	Other relevant outcomes
Heemskerk *et al.* [51]	Sepsis	bIALP ($n = 11$), placebo ($n = 5$) (AKI patients)	Improved CCL within 7 days	Reduced renal oxidative stress
Kats *et al.* [57]	Cardiothoracic surgery	bIALP ($n = 32$), placebo ($n = 31$)	None	Lower incidence of severe systemic inflammation in ALP group
Pickkers *et al.* [13]	Sepsis AKI	bIALP ($n = 16$), placebo ($n = 19$)	Stronger recovery of CCL from day 0 to 28	NA
Pickkers *et al.* [52]	Sepsis AKI	RecALP ($n = 111$), placebo ($n = 116$)	Significantly stronger improvement of CCL from day 0 to 28	Negative primary outcome (improvement of CCL within 7 days) 28- and 90-day mortality were significantly lower in ALP group
Keizer *et al.* [58]	Cardiothoracic surgery	bIALP ($n = 27$), placebo ($n = 26$)	None	Inconclusive
Steenvoorden *et al.* [55]	Kidney transplantation – feasibility pilot	bIALP ($n = 5$), placebo ($n = 6$)	Lower expression of urinary kidney injury biomarkers on day 7	NA
REVIVAL [53]	Sepsis AKI	RecALP, placebo ($N = 649$)	Decreased major adverse kidney events by day 90	Terminated early due to futility on primary outcome; 28-day mortality

RecALP: recombinant ALP; bIALP: bovine intestinal ALP; CCL: creatinine clearance.

Clinical evidence

So far, ALP supplementation has been studied in seven randomized placebo-controlled trials, which are summarized in Table 2. Of the five studies that reported on renal outcomes, four were conducted in patients with Gram-negative sepsis in an intensive care unit setting and one in kidney transplantation.

The first sepsis study, which was conducted in 2009 with 36 patients (15 patients had AKI), showed reduced renal oxidative stress markers and improved creatinine clearance in the ALP-treated patients with AKI compared with patients with AKI treated with placebo [51]. These results prompted a follow-up study with 36 patients (all of whom had SA-AKI), which showed a significantly stronger recovery of endogenous creatinine clearance (ECC) in the ALP-treated group compared with placebo [13]. In the second follow-up study, conducted in 227 patients with SA-AKI, ECC recovery in the first 7 days of AKI was not stronger in the ALP group. However, from day 0 to 28, this recovery was stronger and 28- and 90-day mortality were significantly lower in the ALP group [52].

A larger follow-up trial has since been conducted to confirm the reduced mortality rate at day 28. This trial was terminated after 649 inclusions, in accordance with the results of a futility analysis. However, a significant reduction in the need for dialysis in the ALP-treated group was described by the researchers [53].

The studies evaluating the use of ALP as a treatment for SA-AKI patients were well executed but have not demonstrated definitive clinical benefit. A conclusion that ALP treatment of AKI is therefore ineffective should be nuanced for two reasons. In

general, clinical trials of sepsis are complicated by heterogeneity that cannot be predicted using clinical data, which makes it difficult to establish treatment effects [54]. Furthermore, most *in vivo* trials have evaluated the effects of ALP administration prior to or shortly after LPS infusion, which could mean that administering ALP after AKI has already developed is simply too late. The latter was also hypothesized by Pickkers *et al.* [52], who showed that a longer time to treatment was related to an increased mortality risk.

The factors involved in inducing AKI are in themselves multifactorial, especially in SA-AKI [1], and determining on an individual level what exactly is going on inside the kidney is arduous. Which is why the use of kidney injury biomarkers—in addition to serum creatinine sampling and urine output measurements—has been proposed as a means to help identify different AKI pheno- and endotypes. It is likely that the correct identification of these pheno- and endotypes will become an essential part of the search for AKI treatment strategies [1]. For ALP treatment specifically, biomarkers could be used to identify AKI subtypes in which true ALP substrates—such as extracellular nucleotides—are crucial to the extent of kidney damage.

The mechanisms described in the present review suggest that ALP can best be used to redirect cell–cell signalling away from damaging patterns, which are an essential part of AKI pathophysiology. With the aim of finding a population with a relatively homogeneous insult to the kidney, our group conducted a feasibility pilot study with ALP supplementation to combat IRI in kidney transplantation. Although many of the proposed effects of ALP are related to the immune system and kidney transplantation is always combined with the use of immunosuppressive drugs, it can be assumed that there is high potential for altered purinergic signalling in this setting (including the reduction of nephrotoxicity by these same immunosuppressants) [30].

Five recipients of a living-donor kidney were treated with ALP and six with a placebo. Measured glomerular filtration rate at 1 year after transplantation did not differ between groups. However, the expression of inflammatory kidney injury biomarkers CCL14 and neutrophil gelatinase-associated lipocalin were lower in the ALP-treated group [55]. This matches the hypothesis that intrarenal inflammation might be reduced by ALP but does not confirm it. A larger follow-up trial in deceased-donor kidney transplantation (where the extent of IRI is much greater than in living-donor kidney transplantation) has been initiated (EudraCT: 2021-006767-14).

To understand the true renoprotective potential of ALP, future trials need to evaluate the barrier hypothesis to determine if ALP supplementation can prevent tubular and vascular injury directly. Clinical trials should aim to treat patients before or as soon after the insult as possible. Cardiothoracic surgery, kidney transplantation and, in specific cases, the use of nephrotoxic drugs form the ideal settings for this. Additionally, the use of outcome criteria that directly reflect the extent of kidney damage is recommended.

CONCLUSIONS

The endogenous enzyme ALP has an intricate connection with the host-response to systemic insults and with the preservation of natural barrier tissues. Despite promising *in vivo* results, clinical trials have yet to conclude if these effects translate to tangible improvement of patient health. Renewed insights into the interaction of exogenous ALP with TLRs and purinergic signalling, and its function in the protection of barrier tissues and the activity of endogenous ALP, can help guide new and ongoing trials.

FUNDING

This study was supported by a TKI-PPP grant to Amsterdam UMC (to L.V.) by Health Holland B.V. and Alloksys Life Sciences.

AUTHORS' CONTRIBUTIONS

T.S.S. drafted the original manuscript under supervision of L.V. and J.A.J.R., who also reviewed and edited the manuscript. J.W.H. conceptualized the project and reviewed the manuscript. F.J.B., H.G.D.L. and R.A. reviewed and edited the manuscript.

DATA AVAILABILITY STATEMENT

No new data were generated or analysed in support of this research.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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Received: November 2, 2023; Editorial decision: January 22, 2024