Developing the first treatment for ACUTE KIDNEY INJURY

Acute Kidney Injury (AKI) is a devastating disease characterised by a rapid loss of kidney function resulting in inability to maintain fluid, electrolyte and acid-base balance. AKI is particularly common in patients admitted to the Intensive Care Unit where incidence rates between 22% to as high as 67% of admissions have been reported[1,2]. Even relatively small changes in serum creatinine are related to worse outcomes in AKI patients. Depending on the severity of kidney injury, the mortality rate can be as high as 80%. Moreover, a third of all patients who survive an episode of AKI develop chronic kidney disease, which is accompanied by an enormous burden to both patient and healthcare providers[3,4]. Despite an estimated 2 million AKI patients in the Western world per year, there are no drugs approved for the treatment of AKI.

By Tim Knotnerus

Acute Kidney Injury (AKI) is a complex multifactorial disease with inflammatory, ischaemic, necrotic and apoptopic components simultaneously occurring, which rapidly cause damage and functional failure of the kidneys. Sepsis is the most common cause of AKI, accounting for approximately 50% of cases, and sepsis-associated AKI (SA-AKI) is associated with short- and long-term risk of death[5-7]. During sepsis, the initial host response to an infection becomes amplified and then dysregulated, bringing the patient into alternating hypo- and hyper-inflammatory states. Several measures can be taken primarily to preserve haemodynamics in SA-AKI patients, including volume resuscitation and the use of diuretics, vasopressors and inotropes, which could have negative effects on the kidney. Once AKI develops, there is no specific treatment and only general supportive measures are available. A new therapy would thus be very welcome and should be aimed at targeting both inflammation and renal function impairment.

Renal replacement therapy (RRT)

Currently, critically ill AKI patients are only offered supportive care including the use of renal replacement therapy (dialysis) to allow the kidneys to functionally recover. Supportive care is of limited benefit because it does not improve or affect markers such as fluid balance, electrolytes and acid-base balance. Dialysis is also associated with a number of problems such as the unwanted removal of minerals and vitamins as well as disrupting dosing and control of drug levels. Indeed, there is no

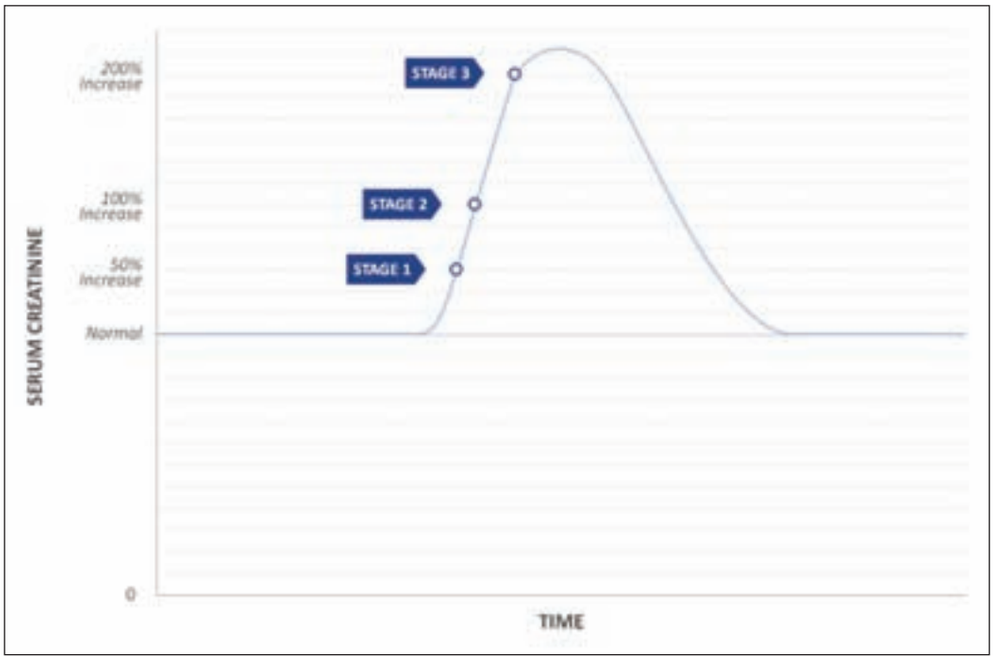


Figure 1a
The most widely-used biomarker for AKI is serum creatinine, a breakdown product from muscle tissue that is normally removed by the kidney. Serum creatinine levels are stable in healthy individuals but a sudden increase is observed in an AKI setting. A 50-100% relative increase in serum creatinine is the threshold that defines stage 1 AKI (or a 0,3mg/dl or 26umol/L absolute increase or a decreased urine output). A further increase of 100-200% and then >200% in serum creatinine correspond to AKI stages 2 and 3, respectively

standard protocols or set of criteria to guide RRT intervention and the best time to start RRT is controversial because the only studies linking timing with outcome are observational. Once RRT is started, uncertainty exists about when to stop[8]. Furthermore, no suitably powered randomised controlled trials have been done to address the question on modality, ie intermittent or continuous forms of RRT. The appropriate intensity of RRT is also uncertain, especially in critically ill patients, as indicated by two landmark studies conducted by the Veterans Affairs/National Institutes of Health (VA/NIH) Acute Renal Failure Trial Network (2008)[9] and The Renal Replacement Therapy Study Investigators (2009)[10]. These concluded that intensive renal support in critically ill patients with AKI did not decrease mortality, improve recovery of kidney function, or reduce the rate of non-renal organ failure, as compared to less intensive regimen similar to usual-care practices.

Therapeutic strategies

Despite the significant unmet medical need and associated commercial potential for new treatments of AKI, especially SA-AKI, no pharmacological treatment is currently approved and only a limited number of candidate drugs or biological therapies are in clinical development. The multifactorial pathogenesis of the disease probably necessitates a multifactorial intervention to allow adequate response and durable restoration of kidney function, which limits the number of potentially efficacious treatment options.

The only anti-inflammatory biological with such a dual mechanism of action, which also showed significantly improved kidney function in two Phase IIa trials, is Alkaline Phosphatase (AP). This membrane bound homodimeric enzyme exists in humans as four different isoenzymes referred to as placental, germ cell, intestinal, or tissue non-specific AP. AP was originally considered as a novel therapy for sepsis because it dephosphorylates and detoxifies endotoxin (lipopolysaccharide, LPS).

From the first Phase IIa trial, conducted in 36 ICU patients with sepsis, it became clear that infusion of a purified bovine form of AP displayed clinical activity particularly in those patients who were at risk to develop or had already been diagnosed with AKI[11]. The treatment's ability to intervene in AKI may be due to AP's dual mechanism of action via dephosphorylation of both LPS and endogenous inflammatory triggers such as extracellular ATP and ADP[12]. Dephosphorylating ATP and ADP to Adenosine, by AP, inhibits formation of platelet-neutrophil aggregates, inhibits leukocyte endothelial adhesion, transmigration and inhibits inflammatory activation. Improvement of cellular stress and damage markers, and apoptotic proteins, indicate that this local anti-inflammatory effect is due to better control of the damage response in peritubular and tubular cells. To confirm the beneficial effects of AP, a second Phase IIa

study was conducted, which focused specifically on patients with sepsis and AKI. In this study, 36 sepsis-associated AKI patients received either bovine AP or placebo intravenously for 48 hours[13]. AP treatment resulted in improvement of kidney function, as measured and demonstrated by a significantly faster recovery of creatinine clearance, and displayed a strong trend towards reduction in requirement and duration of dialysis. In addition, AP-treated patients showed a significantly reduced length of ICU stay and demonstrated signs of improvement of other organs as exemplified by a trend towards reduced need for mechanical ventilation.

During the course of the study, both renal damage biomarkers (urinary levels of KIM-1 and IL-18) and anti-inflammatory biomarkers (blood levels of CRP, LBP, and IL-6) declined significantly faster and were maintained at low levels in the AP treatment group. This strongly suggested that infusion of AP not only exerted local efficacy in failing kidneys but also has systemic anti-inflammatory properties. This clinical activity is corroborated by the efficacy of AP in multiple preclinical animal models of kidney injury, whether induced by ischaemia-reperfusion injury or by endotoxin. The physiological response to treatment with AP follows a very clear pattern by serum biomarkers and histopathology: an immediate local suppression of inflammatory processes in the microcirculation and underlying tissue (apparent by reduced leukocyte activation and extravasation, and reduced inflammatory cytokine production), accompanied by suppression of acute damage to the peritubular tissues. Within 24 hours this resulted in sustained renal blood flow, perfusion, creatinine clearance and GFR.

The Dutch company AM-Pharma is translating the use of AP to clinical application in AKI patients and has produced a recombinant human form of AP (recAP) with improved characteristics compared to purified bovine AP, including enhanced stability and half-life. recAP has recently been tested in a Phase I trial in healthy volunteers, showing a clean safety profile and AM Pharma will soon start a large Phase II trial in sepsis-associated AKI patients in the US and Europe.

Another therapeutic programme in clinical development for AKI involves allogenic mesenchymal stem cell therapy for the treatment of ischaemia-reperfusion injury-induced AKI. The stem cells are derived from pluripotent cells resident in the bone marrow, processed and expanded *in vitro*. The primary mode of action is thought to be via paracrine and endocrine mechanisms because engraftment of the stem cells after differentiation into target cells is absent or rare and fusion of these mesenchymal stem cells with renal cells is not observed[14]. Efficacy of stem cell therapy will depend on the expression of both trophic actions via secretion of growth factors such as VEGF, HGF, IGF-1 and SDF-1, as well as anti-inflammatory actions via chemokine and cytokine production and increase of adhesion molecule expression[14]. In order to avoid

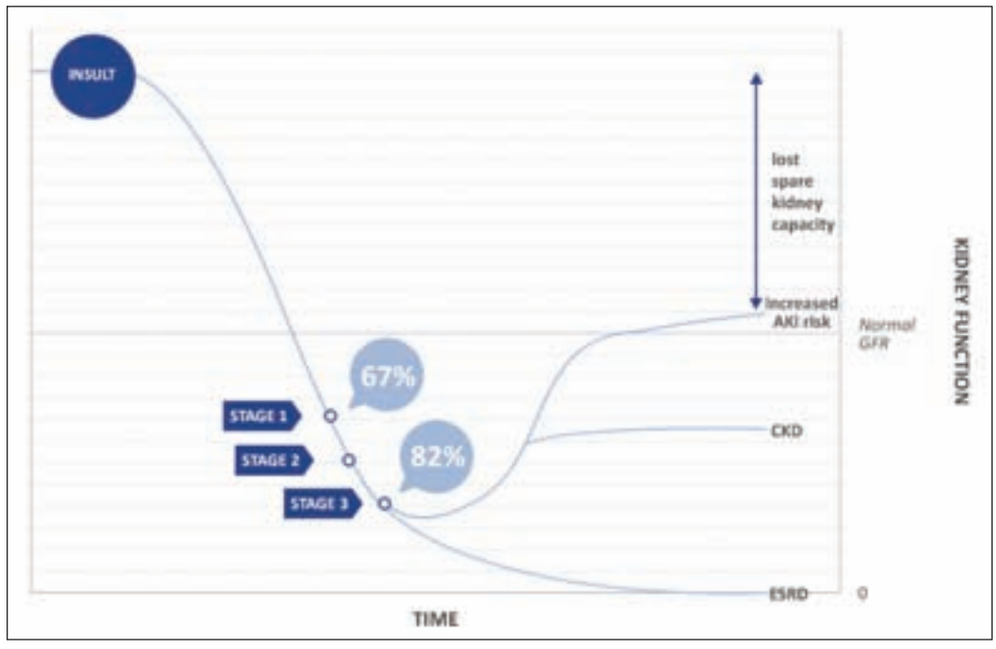
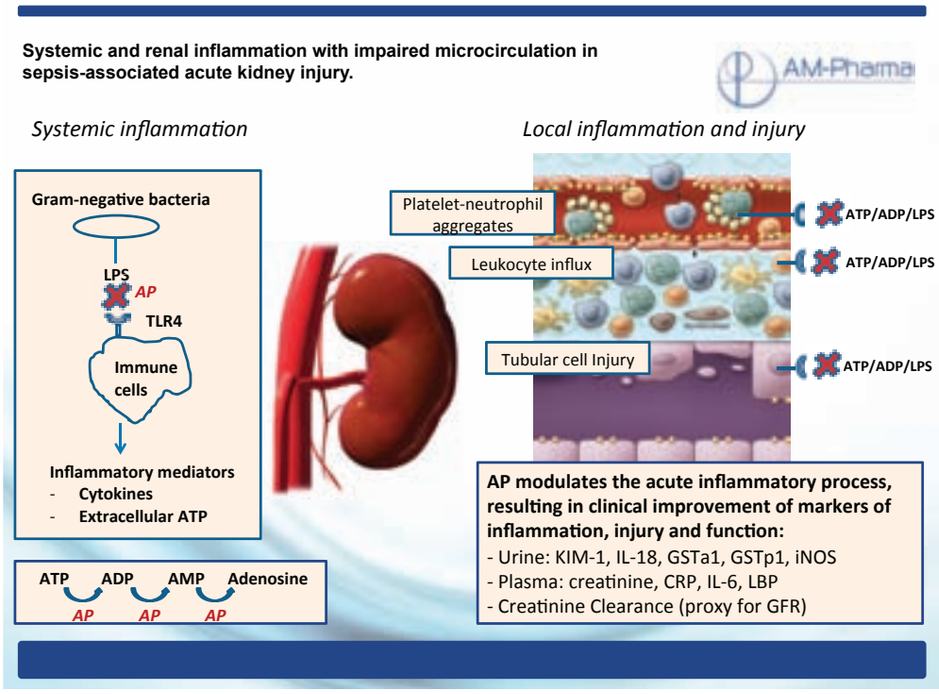


Figure 1b
Patients who have an AKI insult lose kidney capacity as measured by glomerular filtration rate (GFR). By the time an increase of serum creatinine can be measured, already 50% of the kidney capacity has been lost, progressing to 82% of lost kidney capacity at AKI stage 3. Serum creatinine levels in patients who survive an episode of AKI typically return to normal. However, they might have lost a significant portion of their kidneys' spare capacity, which predisposes them to increased risk of subsequent episodes of AKI, chronic kidney disease (CKD) or end stage renal disease (ESRD) in which patients are on permanent dialysis or require kidney transplantation



AP interacts with both the inflammatory and hypoxia pathway in AKI patients

Systemic Inflammation:
Alkaline Phosphatase (AP) dephosphorylates lipopolysaccharide (LPS), thereby antagonising Toll-like receptor 4 (TLR4) and preventing both systemic and renal inflammatory response. Extracellular adenosine triphosphate (ATP) may be converted by AP into tissue-protective and anti-inflammatory adenosine.

Local Inflammation and Injury:
Pathogens and inflammatory mediators (LPS and extracellular ATP) up regulate platelet-neutrophil aggregation, resulting in leukocyte transmigration into the interstitial compartment. This in turn causes swelling, coagulation activation and vasoconstriction, which impairs microcirculation and causes hypoxia, all leading to kidney injury.

Figure adapted from Bonventre et al, Journal of Clinical Investigation 2011[18] and Peters et al, Am J Kidney Dis 2014[19]

flooding of pulmonary circulation with large numbers of mesenchymal stem cells, the administration route of the cells is through the distal thoracic aorta, accessed through the left carotid or femoral artery. For specific homing to the site of injury the cell therapy appears to rely on the expression of CXCR4 and CD44, since their respective ligands SDF-1 and hyaluronic acid are upregulated in AKI. Earlier attempts to use cell-based therapies for the treatment of AKI have failed, mostly due to unexpected side-effects. The US company Allocure is currently developing stem cell therapy for AKI, seemingly justified by the hypoimmunogenic nature of mesenchymal stem cells and its related use in allogenic protocols. From the company's Phase I study in 16 cardiac surgery patients who were at risk of developing AKI, the stem cells appeared safe and well tolerated. Allocure noted a trend towards lower AKI incidence, reduced length of hospital stay and reduced hospital readmission rates compared to a cohort of historical controls. The company is currently conducting a Phase II trial in 200 ischaemia-reperfusion injury-induced AKI patients.

Prevention strategies

From a trial design perspective, there are arguments to developing a prophylactic approach for AKI due to the known medical interventions (timed insult) that may lead to AKI. It is therefore possible to design trials in which treatment can be started prior to such medical interventions. The relatively low incidence rate, however, requires a substantial number of patients to be included in the trial, to ensure a sufficient number of AKI events. This is a clear downside of prevention trials and most prevention studies appear to be underpowered.

Another novel experimental approach for the prevention of AKI after ischaemic reperfusion

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involves the use of selective bone morphogenic protein (BMP) receptor agonists. BMP proteins are closely related to the transforming growth factors (TGF), which are known to induce intracellular signalling through Smad proteins. Expression of the BMP receptor activin-like kinase 3 (Alk3) is elevated early in diseased kidneys after injury[15]. Its deletion in the tubular epithelium leads to enhanced TGF-β1-Smad signalling, epithelial damage and fibrosis, suggesting a protective role for Alk3-mediated signalling in the kidney. In mouse models of kidney disease, BMP receptor agonists selectively triggering Alk3 showed a potential benefit in controlling renal fibrosis. In contrast to their potential fibrotic effects, little is known about the role of BMPs on inflammation, which, as previously discussed, is pivotal in the pathophysiology of AKI. The Canadian company Thrasos is developing small peptide BMP receptor agonists and has completed Phase I studies demonstrating safety and useful pharmacokinetic profiles. A Phase II trial is currently ongoing in patients scheduled for cardiac surgery considered at increased risk to develop AKI. In this study, treatment will be administered by four IV infusions, the first administration prior to surgery.

An alternative option currently tested to prevent AKI after cardiac surgery is the use of melanocyte-stimulating hormone (MSH), with potent anti-inflammatory activities. A modified alpha MSH-peptide analogue is in development targeting both systemic inflammation and apoptosis caused by hypoxia occurring during surgery. *In vitro* target characterisation indicated that the peptide acts as an agonist on the melanocortin-receptor subtypes 1, 3, 4 and 5[16]. The modified peptide was originally developed by Action Pharma, which has been acquired by AbbVie. The peptide was tested in two Phase IIa studies, the first of which tested the compound in 42 patients to assess potential efficacy on cardiac surgery-induced systemic inflammation. Safety and tolerability were reported but no data on plasma concentration and exposure were published. In the second study, the candidates were administered at two dose levels during surgery and early in the post-operative period.

The trial focused on prevention of AKI and systemic inflammatory response in patients undergoing cardiac surgery on cardiopulmonary bypass. Although the primary endpoint, ie an absolute change in serum creatinine levels compared to baseline within the first seven days after surgery, or until discharge from hospital, was missed, the company reported positive data on a composite endpoint of death, dialysis and kidney function during a 90-day follow-up. The compound is currently being tested in a Phase IIb study.

Current guidelines for AKI essentially state that there is no evidence for the utility of diuretics in preventing or treating AKI. Nevertheless, PLC Medical Systems' RenalGuard, a medical device which is designed to rapidly remove contrast dyes that are known to be toxic to the kidneys, is being sold in Europe. The company's position is that having high urine output does prevent contrast nephropathy provided that efforts are made to prevent dehydration. They do so by using a closed-loop fluid management system comprising a high-volume fluid pump and an intravenous urine collection system that interfaces with a standard Foley catheter. An automated match performed in real time reduces the side-effects associated with either under-hydration or over-hydration. In an investigator-sponsored study in Europe, RenalGuard reduced contrast-induced acute kidney injury from 25% in the control group to 11% in the RenalGuard group[17]. RenalGuard's pivotal study is currently ongoing in the US. A number of other studies related to the use of diuretics is also under way, including a research study testing whether protocolised Diuretic Strategy (ProDiuS), a plan for adjusting diuretic doses based on measured urine output, will improve clinical care for patients with cardiorenal syndrome.

Conclusion

AKI is a devastating disease with high incidence and mortality rates. Sepsis is the most common cause accounting for approximately 50% of cases. Despite the enormous unmet medical need, to date no pharmacological therapy has been licensed to treat or prevent AKI. As a consequence, only supportive care is currently being offered to these critically ill patients, renal replacement therapy being the main option which allows the kidney time to heal but is also associated with a number of problems. New therapies would thus be very welcome. Developing new treatments for this indication is particularly challenging due to the multifactorial pathophysiology of the disease including inflammatory, ischaemic and direct nephrotoxic insults acting simultaneously to rapidly cause functional failure of the kidney. Fortunately, following successful earlier studies, a number of large clinical trials with interesting new approaches are under way. DDW

Tim Knotnerus *joined the AM-Pharma team in 2012 and has since been responsible for Business Development of the company. Previously, Tim was a senior associate at Aescap Venture which he joined in 2008. He was instrumental in the financing and support of various portfolio companies including Avantium (the Netherlands), Biocartis (Switzerland), i-Optics (the Netherlands), Ethical Oncology Sciences, now Clovis, (Italy) and to-BBB (the Netherlands). Tim gained both a Science and Innovation Masters and a Drug Innovation Masters with honors from Utrecht University.*

Alkaline Phosphatase and AM-Pharma history

Alkaline Phosphatase (AP) is an enzyme that is naturally present in many organisms, but is reduced in inflammatory indications such as Acute Kidney Injury (AKI) , Inflammatory Bowel Diseases (IBD) and other conditions. In the early 1990s Professor Poelstra and his group at Groningen University in the Netherlands discovered that one of the functions of this enzyme is to protect organs against inflammation and tissue damage via dephosphorylation of LPS and other potentially harmful substances. In 2001, AM-Pharma licensed an AP use patent from Groningen University and started developing AP for inflammatory indications. AP isolated from calf intestines (bovine AP) was successfully tested in two phase II studies in sepsis and AKI patients and one Phase II study in moderate to severe Ulcerative Colitis patients. As a result of regulatory advice, conversations with potential partners and future supply logistics, the company decided to develop a fully human recombinant form of AP, not sourced from animals but produced using a controlled biotechnological manufacturing methods.

In 2011, AM-Pharma raised €29 million from an investor syndicate including the venture arms of AbbVie and Shire as well as six seasoned venture capital funds. With the newly-raised capital, AM-Pharma went on to develop a proprietary recombinant AP (recAP) which is highly stable and active, and has been optimised for treating inflammatory conditions. It is being developed as an injectable for the treatment of AKI and as an oral formulation for the treatment of Ulcerative Colitis. The enzyme has been produced by GMP manufacture for preclinical and clinical trial supply and commercialisation. The company has recently completed a Phase I study in healthy volunteers with varying doses of recAP showing a clean safety profile and favourable pharmacokinetics. A large, proof-of-concept and dose-finding, Phase III-enabling Phase II study in sepsis-associated AKI patients will start in 2014.

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