Targeted protection of proximal tubular cells by nanoparticle-enhanced delivery of a TLR9-antagonist

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The kidney target site for injury that leads to acute kidney injury (AKI) is the proximal tubule. Nanoparticle-encapsulation enhanced delivery of a selective Toll-like receptor 9 antagonist to mouse proximal tubules and attenuated experimental ischemia-reperfusion injury in a mouse model of AKI.

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The syndrome of acute kidney injury (AKI) has many causes, with sepsis, hypoperfusion, and nephrotoxic injury being the most common. The pathophysiology and clinical features of AKI arise from both the specific direct kidney insult and the local renal and systemic response.

Although negative clinical trials are prevalent in the AKI literature, the failure to translate effective experimental therapy into clinical success results from identifiable factors. The first can be characterized as an "all roads lead to Rome" approach, in which almost all causes of AKI are lumped together, and a universal treatment, based on a mechanism usually identified using an ischemia-reperfusion injury, is applied. A second factor is our limited understanding of human AKI. This limitation is especially obvious for sepsis-related AKI, a common cause of kidney injury, arising from a complex combination of microorganism-related nephrotoxins, the innate immune response, and hypoperfusion at the microcirculatory level combined with reduced large-vessel resistance. This complex array of mechanisms was previously attributed solely to hypoperfusion. We now appreciate that kidney injury can occur under conditions of high as well as low blood flow and that damage-associated molecular patterns and pathogen-associated molecular patterns play an important role. Similar considerations apply to clinical ischemia-reperfusion injury, for which an initiating hypoxic or ischemic event is not always discernible.

A fundamental problem limits our understanding, namely, that a realistic concept of human AKI—kidney cellular injury arising through various mechanisms—conflicts with the consensus function–based definition used for diagnosis, and that is actually a measure of severity. This conflict results in an inevitable delay to a point beyond therapeutic utility in application of treatments designed to prevent injury or act early after injury and now drives the need to incorporate biomarkers of injury into the pure functional definition of AKI. A further consideration is that most treatments are identified and adopted based on studies of how to prevent or treat AKI in otherwise healthy young animals. Generalizing treatment from such models is problematic, given that, at least in developed nations, most patients are older, have multiple comorbidities, including most frequently chronic kidney disease (CKD), or perhaps subclinical CKD, despite a serum creatinine level within the normal range. These factors predispose these patients to greater kidney injury.[1] Indeed, in the absence of a measure of renal functional reserve or of biomarkers that reflect ongoing injury, it is unwise to assume "normal" kidney parenchyma, particularly in elderly subjects with significant vascular disease, such as those undergoing cardiopulmonary bypass.[2] Superimposed insults amplify injury in such damaged kidneys, which may also respond differently to treatments that are effective in healthy kidneys.[1] Other factors contributing to poor translation of experimental AKI-derived strategies are described elsewhere.[3]

So what should be the targets for treatment in AKI, how should we make the diagnosis, and when should we implement treatment?

Appropriate intervention requires not only diagnosis but also insight into when to intervene in a particular injury pathway. Clearly, the diagnosis of injury needs to be made by injury biomarkers, not markers of function, such as creatinine. Although the latter may be useful in the timing of interventions, such as dialysis, that are based on symptoms, creatinine clearly has little or no role in early diagnosis. There will likely be a role for direct, real-time glomerular filtration rate measurements once these become available. However, there is little delay incurred when diagnosing injury using kidney-damage biomarkers. The stage has been set with the current availability of many novel urinary and circulating biomarkers, such as urinary cell cycle inhibitors (tissue inhibitor of metalloproteinase 2 [TIMP-2] and insulin-like growth factor binding protein 7 [IGFBP-7]), urinary neutrophil gelatinase-associated lipocalin, and urinary kidney injury molecule-1 (KIM-1), to detect kidney injury within hours of a relevant insult (exposure). However, successful clinical

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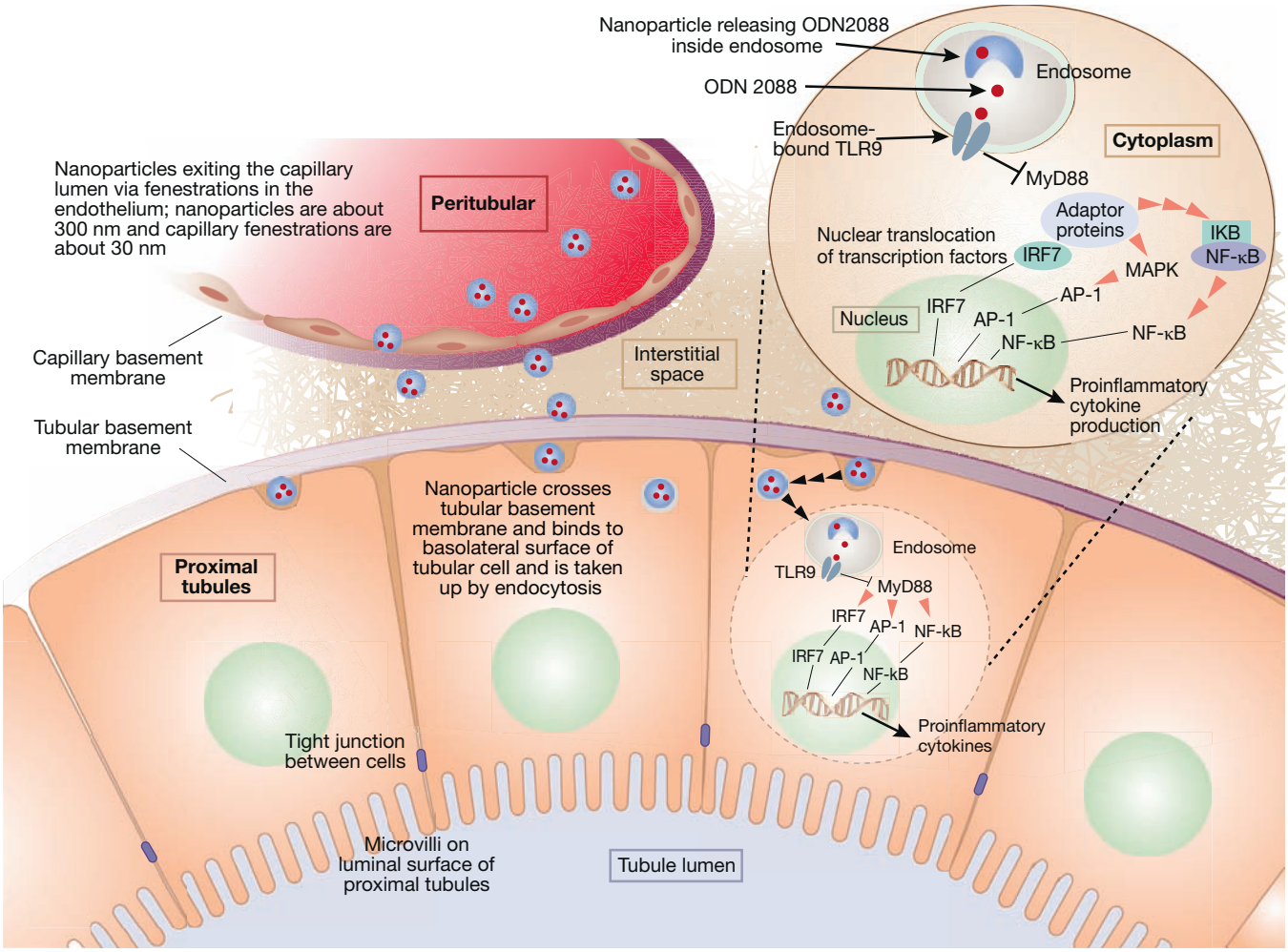

Figure 1 | Possible schema for selective nanoparticle-enhanced drug delivery to proximal tubule. Nanoparticles deliver ODN2088 to proximal tubular cells. After endocytosis and particle breakdown, the ODN2088 binds and inhibits cytosolic Toll-like receptor 9 (TLR9). The mechanisms of uptake, ODN release, and nanoparticle clearance have not been determined. The nanoparticles provide a 30-fold enrichment for delivery of ODN2088 to proximal tubule versus other cell types. Nanoparticles are shown in blue, TLR-9 in orange, and ODN2088 in red. MAPK, mitogen-activated protein kinase; NF-κB, nuclear factor κB.

application of renal injury biomarkers will take time and progressive refinement, just as the application of cardiac ischemic biomarkers evolved over more than 4 decades of clinical use. Even with appropriate timing, most interventions will come after injury, so any treatment for diagnosed AKI needs to provide benefit after injury has occurred. This treatment needs to be a means to either stop progressive injury or accelerate recovery. Successful treatment to target prevention in high-risk groups will be a high-value clinical target.

The appropriate treatment target for most causes of AKI remains the proximal tubule.[4] Although hypoperfusion may mediate hypoxia or ischemia, the fact that hypoxic injury primarily targets the proximal tubule, particularly the S3 segment, was demonstrated definitively many years ago.[5] Elegant experiments confining injury to the proximal tubules have confirmed that proximal tubular injury may cause reversible injury, and that with increasing severity, such injury may lead to progressive fibrosis.[6] Furthermore, both direct proximal tubular injury and secondary injury, such as that accompanying nephron outflow obstruction, lead to disconnection at the glomerulo proximal tubular junction. This consistent feature of CKD[4] provides structural confirmation that injury to the proximal tubule in AKI leads to CKD, regardless of the primary insult. Logically then, protective therapy must target the proximal tubule.

Powerful insights into both selective targeting and treatment of proximal tubular injury are provided in the current issue. Han *et al.*[7] used nanoparticle-mediated targeting of proximal tubular Toll-like receptor 9 (TLR9) to attenuate AKI in an ischemia-reperfusion injury (IRI) model. The authors used polymer-based mesoscale nanoparticles (MNP) to encapsulate a selective TLR9 antagonist, unmethylated CpG oligonucleotide, ODN2088, or a negative control ODN. They demonstrated that these localized to the tubules after

intravenous injection with high proximal tubular and overall kidney selectivity, with no MNP localization to other tubular segments, endothelial cells, or glomerular mesangial cells, and with 30-fold kidney selectivity over other organs, including lung, liver, spleen, and heart. Mice treated with the TLR9 antagonist at the time of reperfusion or 1.5 hours later were protected against IRI with evidence of attenuated necrosis and inflammation. The need for proximal tubular cellular as well as receptor specificity of the molecular target was highlighted by the absence of protection from IRI in whole-animal TLR9 deletion. By contrast, whole-animal deletion of TLR4, which is also heavily expressed in proximal tubules during IRI and plays a role in injury, is protective.

Kidney selectivity is assisted by high blood flow and has previously facilitated localization of viral vectors and small interfering RNA to the kidney.[8] Effective proximal tubular targeting in the present study was facilitated by nanoparticle encapsulation. The alternative control (naked ODN2088) given 6 hours before ischemia failed to protect against function, histologic injury, neutrophil infiltration, or induction of proinflammatory chemokines and cytokines. These findings raise interesting questions regarding the mechanism of localization of non-filtrable MNPs, with a mean diameter of 312 nm (vs. an effective glomerular pore size of less than 8 nm), how this encapsulated molecule interacts with its receptor, and whether other kidney cell types might be targeted for other conditions. As nanoparticles administered intravenously are rapidly coated by plasma, this will affect handling by various cells and organs. The effective *in vivo* hydrodynamic diameter of the MNPs used by Han and colleagues[7] is unknown. Based on initial size, they are likely taken up initially through the capillary fenestrae and then after traversing the basement membrane, by endocytosis on the basolateral (interstititial) membrane of proximal tubular cells. Within these cells, they are presumably cleared by enzymatic cleavage with intracellular release of the ODN to reach its cystolic target, the TLR9 receptor, which may be inserted into the endosome as schematically illustrated in Figure 1 (c.f. Donahue and Wilhelm[9]). Clearly, this schema requires validation.

The TLR9-based protection demstrated by Han and colleagues[7] is mechanistically revealing and may become a useful preventative strategy. However, for reasons already discussed, this protection may not be useful in an established cascade of injury after AKI has been initiated. Although antagonism of TLR9 was protective 1.5 hours after injury, this protection seems unlikely to be clinically helpful in established AKI. However, the finding that it is possible to provide relatively specific drug delivery to the proximal tubule remains of major potential importance. The results support the notion that nanoparticle technology can generate useful "vehicles" to facilitate delivery of relevant agonists or antagonists targeting an array of pathways involved in proximal tubular injury. Not only the site of injury may be targeted, but also the specific pathway involved in the context of the specific cause(s) of injury, for example, with different targets in sepsis-AKI, contrast-AKI, hypoperfusion-AKI, etc.

Of course, many challenges remain. Understanding how these nanoparticles are delivered to kidney tubular cells is important. Will this delivery system work in kidneys with varying degrees of underlying CKD, hypoperfusion, and microcirculatory rarefaction, or in the presence of extensive inflammatory cell infiltration? Does underlying CKD, whether subclinical or overt, change the cellular and global kidney response to nanoparticle drug delivery, as occurs after superimposed injury?[1] Is delivery linked to renal functional reserve? Can other proximal tubule receptors and pathways be targeted? Most importantly, are such large MNPs themselves injurious as they accumulate? Obviously, there are presently many more questions than answers. However, the promise of cell-specific drug delivery to proximal tubular cells is an exciting and powerful step forward in applying a concept akin to precision medicine to kidney disease.

DISCLOSURE

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