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Heparanase in Acute Kidney Injury

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Abstract

Recent years have brought about fledgling realization of the role played by heparanase in the pathogenesis of diverse diseases including kidney diseases and, specifically, acute kidney injury. Human heparanase-1 is critically and uniquely engaged in cleavage of heparan sulfate, an integral part of glycocalyx and extracellular matrix where it harbors distinct growth factors, cytokines, and other biologically active molecules. The enzyme is induced and activated in acute kidney injury regardless of its causes, ischemic, nephrotoxic, septic or transplantation-related. This event unleashes a host of sequelae characteristic of the pathogenesis of acute kidney injury, such as induction and reinforcement of innate immune responses, predisposition to thrombosis, activation of monocytes/macrophages and remodeling of the extracellular matrix, thus setting up the stage for future fibrotic complications and development of chronic kidney disease. We briefly discuss the emerging therapeutic strategies of inhibiting heparanase, as well as the diagnostic value of detecting products of heparanase activity for prognostication and treatment.

Keywords

Inflammation; Coagulation; Renal disease; Kidney transplantation; Renal ischemia

1 Introduction

Acute kidney injury (AKI), aka as “acute renal failure” or “acute tubular necrosis”, is a syndrome of acute renal functional impairment of varying severity which, depending on the nature of the insult –nephrotoxic, septic, or ischemic – may or may not be accompanied by the commensurate morphologic changes [1]. The causes of this syndrome could be pre-renal (i.e., cardiac failure or extracellular volume depletion), post-renal (i.e., blockage of the urine outflow), and intrinsic renal (i.e., toxic, septic or ischemic). All three categories of diseases, acutely affecting renal functions, invariably injure the kidney by a combination of hemodynamic and tubular alterations differing in their intensity. Acute pre-renal injury to the kidney affects predominantly renal circulation with the attendant tubulopathy. Acute post-

renal injury to the kidney affects tubules with the attendant renal circulatory abnormalities. Acute intrinsic kidney injury affects both to a variable degree. The functional coupling of these two pathogenic mechanisms has been emphasized by H. Valtin [2], “both the renal vasculature and the tubular system run in series and are intertwined both anatomically and functionally, initial damage to the tubules will quickly involve the vessels, and vice versa.” Despite such anatomic and functional coupling, comparative analysis of the transcriptome in mouse models of ischemic and volume-depleted AKI (both manifesting with the similar degree of renal failure) showed profoundly distinct gene profiles [3].

The most common morphologic findings include dilation of the tubules and flattening of distal and proximal tubular epithelium with the desquamation of proximal epithelial cells, presence of granular and brownish-reddish casts, focal infiltration and edema of the proximal tubular epithelium and the interstitium, mitotic figures in the proximal and distal epithelial cells, and occasional tubular necrosis. These modest morphologic abnormalities are in sharp contrast to functional failure. The latter is characterized by a precipitous drop in glomerular filtration rate exceeding the reduction in renal blood flow, increased proximal tubular and interstitial pressure, thus counteracting the filtration pressure [4].

The incidence of AKI has been steadily increasing, especially among elderly hospitalized patients and critically ill subjects and may escalate in ICU to 78% [5, 6]. When developed, AKI is associated with the eight-fold increased mortality [7, 8]. The most vulnerable populations include diabetic patients, groups with other preconditions or surgical interventions and elderly subjects. Subsets of AKI are graft rejection and GVHD, acute-on-chronic, and AKI-CKD continuum.

Given the frequency and implications of this syndrome, rigorous investigations were conducted through years, which yielded, among other important findings, insights into the role of heparanase in its pathogenesis. This particular subject will be the center point of the current brief overview.

1.1 Heparanase Secretion in Stress

The cellular processing of heparanase involves exocytic and endocytic compartments, including early and late exocytic vesicles, endocytic vesicles, and lysosomes. Endocytic traffic of components of glycocalyx, such as heparan sulfate and proteoglycans and their self-assembly into heparan sulfate proteoglycans, as well as the transport of (pro)heparanase, have been previously reviewed [9–11]. This pathway, in addition to lysosomal degradation of endocytosed material, includes recycling of endosomal cargo. Briefly, synthesized pre-pro-heparanase is initially trafficked to the ER, shuttled to the Golgi apparatus and eventually secreted in the form of 65kD pro-heparanase [12]. This latent form of the enzyme interacts with syndecans and undergoes endocytosis. The active form of heparanase is generated by lysosomal cathepsin-induced cleavage of pro-enzyme yielding 50kD and 8kD fragments, both required for its activity. Heparanase has a low-pH optimum of 5.0–6.0 to cleave the glycosidic bond between β -D-glucuronic acid and N-(sulfated)-D-glucosamine preferentially targeting the boundary between highly- and mixed-sulfated regions of HS, thus producing highly sulfated end-domains and yielding mixed sulfated saccharide products of a size sufficient to bind proteins. Synthetic pathways of heparanase partially overlap with

those of HS and MMP-9. This intricate traffic route highlights the mechanisms and sites of possible aberrations under stress conditions during endocytic, lysosomal, and ER transits.

2 ER Stress

Endoplasmic reticulum (ER) is responsible for the storage and release of calcium and folding and secretion of proteins, including heparanase, and biogenesis of lipids. These functions become impaired due to ER stress induced in the kidney by ischemia/reperfusion injury, NSAIDs, aminoglycosides, cisplatin, calcineurin inhibitors, cyclosporine A and tacrolimus, and some heavy metals (cadmium, mercury, lead) [13]. Accumulation of unfolded proteins triggers a default unfolded protein response (UPR). UPR initially affords cytoprotection and, if prolonged, leads to autodestruction. Cytoprotective effects of UPR are accomplished via upregulation of chaperones, glucose-regulated proteins GRP78 and GRP94, by inositol-requiring protein-1 (IRE1) kinase and protein disulfide isomerase (PDI), both assisting in protein folding, and by suppression of translation through phosphorylation of eukaryotic translation initiation factor 2 α , thus reducing protein synthesis and burden of folding. When stress is prolonged these pathways become deactivated triggering pro-apoptotic pathways [14]. In ischemia/reperfusion kidney injury, eIF2 α phosphorylation exerts a renoprotective effect, and overexpression of the chaperone GRP78 protects cultured epithelial cells against hydrogen peroxide, iodoacetamine and 2,3,5-tris(glutathione-S-yl)hydroquinone, all potent oxidants inducing ER stress [15]. It remains to be established how ER stress accompanying AKI affects the processing of pre-pro-heparanase.

3 Lysosomes

Reactive oxygen species, a common companion of cell stress, and many nephrotoxic agents with lysosomotropic effects result in lysosomal membrane permeabilization, often preceding mitochondrial membrane permeabilization and release of cytochrome c [16]. This, in turn, leads to the activation of acidic sphingomyelinase and generation of ceramide, triggering autocatalytic proteolysis by the released cathepsins.

Gentamycin-induced AKI is of special interest, as it is associated with the enlargement of the lysosomal compartment where the antibiotic is concentrated together with phospholipids and the chaperone HSP73, forming myeloid bodies typical of tubular phospholipidosis. Gentamycin binds to and interferes with chaperone activity of HSP73 [17]. Another condition typically associated with the distension of lysosomes and vacuolization and swelling of the proximal tubular epithelia is represented by osmotic nephrosis – AKI initiated by contrast media, dextrans or sucrose [18]. Immunoglobulin G therapy can lead to osmotic nephrosis due to the presence of IgG stabilizers, sucrose, and sorbitol, at 10% each. Volume expanders like low-molecular-weight dextran and mannitol, especially when combined with cyclosporine or furosemide, can result in osmotic nephrosis and AKI. Another volume expander, hydroxyethyl starch, results in about 40% chance of developing osmotic nephrosis and AKI. Ionic and non-ionic hyperosmolar radio-contrast media, first and second generations of iodine-containing contrast agents, in combination with dehydration, are well-known causes of osmotic nephrosis. In all these cases, proximal tubular cells are swollen by accumulating vacuoles, which fuse with the lysosomes but fail

to undergo complete digestion. The above-mentioned loss of pH gradient may have direct consequences for not only the maturation of the enzyme but also its ability to cleave HS. This side of the equation remains poorly investigated.

4 Heparanase Synthesis, Secretion, and Activity in AKI

A few available studies point out at several features of heparanase in AKI (Masola et al., in this volume). Firstly, unbiased microarray analyses of renal transcriptome following AKI did not reveal any dramatic changes in the level of heparanase message in the immediate-early (up to 4 h), early (4–24 h), and intermediate-late (48 h and longer) stages post-injury [19]. A modest increase in heparanase mRNA is observed in mice with ischemic AKI [20, 21] 48 h after the insult, but much higher gene expression is observed in heparanase transgenic (Hpa-tg) mice 72 h after ischemia. In a study of septic mice, the heparanase message level was found to be modestly induced at 4 h and further increased 24 h after initiation of polymicrobial sepsis using colon ligation and puncture model [22]. In either of these conditions, the activity of heparanase was dramatically increased, thus accounting for the elevated levels of HS fragments in the circulation (see below). Importantly, in both cases application of heparanase inhibitors reduced pathologic sequelae of ischemia or sepsis. These findings are consistent with the current view of predominantly epigenetic regulation of the enzyme gene expression [23]. All these findings bring about an unresolved question. In the face of developing lysosomal dysfunction with the collapse of pH gradient, one would expect that impaired processing of pro-heparanase and reduction of heparanase activity would ensue; yet the existing evidence indicates the opposite – an increase in enzymatic activity under stress conditions. It is possible that there exist chronologic shifts in heparanase activity reflecting the state of lysosomal dysfunction and its restoration in the course of a disease.

5 Non-catalytic Actions of Heparanase

Besides its catalytic activity toward HS, heparanase exerts several non-catalytic actions, including endothelial cell migration/invasion, infiltration of immune cells, coagulation, fibrosis, autophagy, exosome pinching, and inflammation. These effects are thought to be mediated by membrane receptors still to be identified [24]. Binding of the pro-Hpa (65 kDa) to these receptors activates downstream signaling which includes Akt, PI3K, ERK, p38 and Src [25–27] (Ilan et al., in this volume). Notably, heparanase can also translocate into the nucleus where it is capable of regulating gene expression (Khanna and Parish, in this volume).

6 Heparanase Actions on Glycocalyx and ECM

The targets of heparanase, HS chains, are either bound to glycocalyx proteoglycan core proteins, like syndecans, or proteoglycans located in the extracellular matrix, like perlecan. Active heparanase, therefore, not only trims HS bound to proteoglycans comprising glycocalyx but also cleaves those attached to the extracellular matrix proteins. It is remarkable how broad is the list of HS-binding proteins; it includes more than 300 interacting partners which can be liberated upon HS degradation [28]. Through the

degradation of HS, heparanase is involved in an array of functions which are mediated by the released biologically active molecules, such as growth factors and cytokines bound to HS, interaction with heparanase-binding proteins and activation of kinases as Src, Akt, p38 MAPK, modulating the activity of FGF-2 and TGF- β signaling pathways, and activation of FGF-2 signaling [29]. One of the important binding partners of HS is albumin, which electrostatically interacts with the glycocalyx. The loss of electronegativity of the surface layer, therefore, is accompanied by liberation of albumin and attached to it endothelial survival factor, sphingosine-1-phosphate. When HS chains abundantly decorate proteoglycans, as it happens in Hpa-knockout mice, sepsis-induced degradation of pulmonary endothelial glycocalyx and endothelial hyperpermeability are inhibited [30]. In contrast, mice overexpressing heparanase exhibit reduced leukocyte crawling, adhesion, and infiltration of inflamed sites, presumably as a result of the defective ability of truncated HS chains in the glycocalyx to ligate chemokines, as well as due to the increase in “heparin-resembling” HS fragments endowed with anti-inflammatory properties [23]. Because HS interacts with integrins, thus participating in cell adhesion and motility, these functions could be critically affected by changes in heparanase activity. Furthermore, both HS and heparanase are important modulators of syndecan-1 and vice versa. Syndecan-1 regulates pro-heparanase internalization and endosomal-lysosomal processing leading to its activation [31]. On the other hand, HS and elevated heparanase activity regulate shedding of syndecans [32]. Yet, the mode of heparanase action in the extracellular environment with a near-neutral pH remains a vexing question.

7 Heparanase and Activation of TLR and Inflammation

One of the hallmark action of heparanase/ HSPGs system is to orchestrate inflammation [33]. Specifically, heparanase plays a pivotal role in the inflammatory response, mainly due its enzymatic activity on ECM and the subsequent release of harbored in it pro-inflammatory cytokines, including IL-2, IL-8, bFGF and TGF- β , and via modulating the interaction between the leucocytes and endothelial cells surface and stimulation of leucocyte recruitment, rolling process and extravasation [34–36]. It should be emphasized that neutrophils and activated T-lymphocytes possess HS degrading activity, features essential for their extravasation and infiltration into inflamed tissues. This degrading activity was later attributed to heparanase [37]. Additional studies have demonstrated that non-enzymatic activities are behind some of the heparanase pro-inflammatory effects such as cell adhesion and signal transduction [12]. Upregulation of heparanase activity/abundance has been detected in endothelial and epithelial cells of inflamed tissues as in chronic colitis, Crohn’s disease, sepsis-associated lung injury, chronic pancreatitis, rheumatoid arthritis, keratoconic cornea, neuro-inflammatory response in subarachnoid hemorrhage (SAH), and Herpes Simplex infection [38–43]. For instance, colitis is characterized by heparanase upregulation in enterocytes, where it promotes monocyte-to-macrophage activation and sustained chronic inflammation [38]. Similarly, chronic and acute kidney injuries induced by I/R sustain inflammatory characteristic as evident by Hpa-induced macrophages polarization via cathepsin L of tubular origin. The mechanisms underlying Hpa-induced macrophage activation is not fully characterized; active heparanase regulates activation of macrophage via TLR2 and TLR4 by HS degradation fragments leading to increased levels of TNF- α .

thereby creating chronic inflammatory conditions fostering macrophage-mediated renal injury and heparanase up-regulation. In this context, it was reported that intact HS inhibits TLR4 signaling and macrophage activation, whereas HS fragments are thought to play a key role in the activation process [44–47]. Experimental model of I/R renal injury is characterized by enhanced levels of heparanase in both tubule and glomeruli [48]. Heparanase then induces tubular cell apoptosis and Damage Associated Molecular Patterns (DAMPs) production. DAMPs, heparanase-released HS-fragments, and molecules generated from necrotic cells activate TLRs both on macrophages and tubular cells. In response to direct hypoxic stimuli and TLR activation, tubular cells produce pro-inflammatory cytokines, which attract and activate macrophages and the presence of high levels of heparanase facilitates M1 polarization of infiltrated macrophages, which worsen parenchymal damage [48]. Recently, we examined whether heparanase plays a role in acute pancreatitis (AP), one of the most common diseases in gastroenterology [49]. In this study (Khamaysi et al., in this volume), we provided evidence that pancreatic heparanase expression and activity are significantly increased following cerulein-induced AP. Moreover, pancreas edema and inflammation, as well as the induction of cytokines and signaling molecules following cerulein treatment were attenuated markedly by PG545 and SST0001 (heparanase inhibitors - see below), implying that heparanase plays a significant role in AP. Notably, all the above features appear even more pronounced in transgenic mice overexpressing heparanase. In line with its pro-inflammatory role, upregulation of heparanase during AP was associated with the recruitment of neutrophils and enhanced expression of various cytokines including TNF α and IL-6, key cytokines implicated in experimental and clinical pancreatic injury [50]. Notably, induction of TNF α increased I κ B phosphorylation and nuclear translocation of p65, which were prominently decreased by PG545.

8 Heparanase and Coagulation

Degradation of HS by heparanase causes remodeling of extracellular matrix (ECM), and release of numerous biologically active molecules including cytokines, growth factors and other sequestered components which activate several physiological and pathophysiological processes including blood coagulation [33]. Concerning the latter, it has been shown that heparanase is involved in hemostasis, as was evident by remarkable upregulation of tissue factor (TF) in both in vitro and in vivo models of overexpression of this enzyme and enhancement of the generation of activated factor X (FXa) [51, 52] (Nadir in this volume). Support for potential linkage between heparanase and TF was derived from leukemic patients, where direct correlation between these two parameters was found [53]. Moreover, the addition of heparanase to primary cultured endothelial cells, which usually do not express TF, caused substantial enhancement of their coagulation activity [53]. Based on these studies, it was suggested that pro-heparanase hitched to cellular HS, where it induces a pro-coagulant effect. Moreover, heparanase interacts with TF pathway inhibitor (TFPI) localized to the cell surface of EC and tumor cells, resulting in dissociation of TFPI and subsequently enhanced cellular coagulation activity [54]. Likewise, by using plasma from patients treated with low molecular weight heparin (LMWH), it was demonstrated that pro-Hpa abolishes the anticoagulant effect of unfractionated heparin and the Factor Xa inhibitory activity of LMWH [51, 55]. Similarly, pro-coagulant effects of pro-heparanase were also

mimicked by a peptide corresponding to its major functional heparin-binding domain (HBD-1, residues Lys158 Asp171) [52]. The anticoagulant activity of heparanase could not solely be attributed to its ability to degrade the heparinoids since this process takes hours and requires low pH. Therefore, non-enzymatic mechanisms underlying the involvement of heparanase in hemostasis are more appreciated, including (i) Heparanase can sequester heparin, (ii) The time course of Hpa-induced heparin-neutralization is faster than that of heparin degradation, and (iii) Active platelet-derived heparanase is practically inactive under physiological pH. Moreover, (iv) it reduces unfractionated heparin to about the size of LMWH, which is still highly active as a cofactor for FXa inhibition by AT, and (v) Heparanase-derived peptide (HBD-1) does not possess any enzymatic activity [51]. Most recently, Bayam et al. [56] demonstrated that increased heparanase levels might be responsible for obstructive prosthetic valve thrombosis (PVT). Furthermore, treatment with unfractionated heparin (UFH) significantly increased circulatory heparanase levels, thus attenuating the treatment efficacy as it may be associated with a high risk of thromboembolism and increased thrombus burden in PVT patients. Collectively, these findings suggest that heparanase can be used as pro-coagulant and a therapeutic antidote against heparinoid anti-coagulant activities. (Nadir in this volume).

9 Heparanase in AKI

In light of the inflammatory, fibrotic and ECM remodeling actions of heparanase, it is not surprising that increasing evidence supports the involvement of this enzyme in the pathogenesis of AKI of various etiologies (Masola et al., in this book). The involvement of Hpa/HSPGs in the pathogenesis of AKI has not been studied in depth so far. Nevertheless, the emerging data linking heparanase and inflammatory responses, suggest a role of this enzyme in AKI pathogenesis. Lygizos et al. [57] found that glomerular heparanase is activated during sepsis and contributes to septic AKI. Specifically, the authors induced polymicrobial sepsis in mice using cecal ligation and puncture (CLP) in the presence or absence of competitive heparanase inhibitors (heparin or non-anticoagulant N-desulfated re-N-acetylated heparin [NAH]). CLP-treated mice revealed early activation of glomerular heparanase with coincident loss of glomerular function, as indicated by increased blood urea nitrogen (BUN) and decreased GFR. Administration of the above heparanase inhibitors 2 h before CLP attenuated the deleterious consequences of sepsis, suggesting that glomerular heparanase is active during sepsis and contributes to septic renal dysfunction via uncharacterized mechanisms. In line with these findings, bilateral renal ischemia/reperfusion (I/R) in syndecan-1 deficient mice resulted in increased initial renal failure and tubular injury compared with wt mice [58]. Macrophage and myofibroblast numbers, tubular damage, and BUN were increased and tubular proliferation reduced in the kidneys of syndecan-1 deficient mice compared with wt mice 14 days following injury, suggesting that syndecan-1 promotes tubular survival and repair in murine I/R injury. Support for this notion is derived from the observation that knockdown of syndecan-1 in human tubular epithelial cells in vitro reduced cell proliferation [58]. Selective binding of growth factors suggests that syndecan-1 may promote epithelial restoration [58]. In an additional study, these authors have shown that within 24 h after renal I/R, HSPGs expressed at the abluminal side of peritubular capillaries are induced to bind L-selectin and the monocyte chemoattractant

protein-1, facilitating monocyte extravasation [59]. In agreement with these findings, these authors showed that early monocyte/macrophage influx was impaired in HSPG2(Delta3/Delta3)xCol18a1(-/-) mice, providing keen evidence for the concept that not only endothelial but also (microvascular) BM HSPGs can influence inflammatory responses. A vicious circle of Hpa-driven molecular events promoting chronic inflammation and renal injury has recently been described [60, 61]. This circle is fueled by heterotypic interactions among glomerular, tubular, and immune cell compartments. It appears that latent heparanase, over-expressed by glomerular cells and post-translationally activated by cathepsin L of tubular origin, sustains continuous activation of kidney-damaging macrophages. Briefly, active heparanase governs macrophage activation via activation of TLR2 and TLR4 by HS degradation fragments [47], leading to increased levels of TNF- α thereby creating chronic inflammatory conditions fostering macrophage-mediated renal injury and reinforcing heparanase up-regulation. Also, an interplay between heparanase and the endothelin system has been established. Most recently, Garsen et al. [62] have demonstrated that activation of ET-1 signaling induces heparanase expression in podocytes thus resulting in damaged glycocalyx, proteinuria, and renal failure in experimental diabetes. It should be emphasized that the endothelin system is activated in several disease states characterized by vasoconstriction, proliferation, inflammation, ECM remodeling, and fibrosis, including CKD and AKI [63, 64]. Notably, all the components of the ET system are upregulated in AKI, and its inhibition attenuates the severity of the disease [65, 66]. These findings allude to the likelihood that a similar interplay between ET-1 signaling and heparanase exists in AKI. In this context, we have demonstrated that hypoxia/reoxygenation (H/R) significantly increased the expression of α -SMA, VIM, and FN, EMT-markers in wild type (WT), but not in Hpa-silenced tubular cells. Noteworthy, EMT was prevented in WT cells by SST0001 treatment. In agreement with the *in vitro* results, I/R induced a remarkable up-regulation of EMT markers in Hpa-tg mice after 48–72 h. In contrast, these observations were absent in WT animals [21] (Fig. 1). Next, we examined the involvement of heparanase in the pathogenesis of ischemic reperfusion (I/R) AKI in a mouse model and the protective effect of PG545, a potent heparanase inhibitor. As expected, I/R induced tubular damage and elevation in SCr and BUN to a greater extent in heparanase over-expressing transgenic mice (Hpa-Tg) vs. WT mice [20]. Moreover, TGF- β , vimentin, fibronectin, and α -smooth muscle actin, biomarkers of fibrosis, and TNF α , IL6 and endothelin-1, biomarkers of inflammation, were upregulated in I/R induced AKI, primarily in Hpa-Tg mice, suggesting an adverse role of heparanase in the pathogenesis of AKI (Fig. 1). Remarkably, pretreatment of mice with PG545 ameliorated kidney dysfunction and the up-regulation of heparanase, pro-inflammatory (i.e., IL-6) and pro-fibrotic (i.e., TGF- β) genes induced by I/R. In an additional study by Masola et al. [48] the cross talk between macrophages and HK-2 renal tubular cells during *in vitro* hypoxia/reoxygenation (H/R) was examined. Furthermore, these authors evaluated *in vivo* renal inflammation, macrophage polarization, and histologic changes in mice subjected to mono-lateral I/R and treated with SST0001 for 2 or 7 d. The *in vitro* studies demonstrated that heparanase sustained M1 macrophage polarization, the release of damage-associated molecular patterns in post-H/R tubular cells, the synthesis of pro-inflammatory cytokines, and the up-regulation of TLRs on both epithelial cells and macrophages. Furthermore, heparanase induced partial EMT in HK-2 renal tubular cells by M1 macrophages, which was abolished by heparanase inhibitor. In agreement with these

findings, inhibition of heparanase *in vivo* reduced inflammation and M1 polarization in mice undergoing I/R injury, partially restored renal function and normal histology, and reduced apoptosis. Taken together, our results demonstrate that heparanase plays a harmful role in the development of renal injury and kidney dysfunction as was evident by EMT (Fig. 1) [29] and macrophage polarization, suggesting heparanase inhibition as a promising therapeutic maneuver for AKI (Masola et al., in this volume). The involvement of heparanase in AKI is of special interest, as AKI may advance to CKD. In this context, heparanase activity/abundance has been also documented in diabetic and nondiabetic proteinuric kidney diseases [67, 68]. Heparanase expression was shown to be upregulated in a number of animal models of renal diseases, including passive Heymann nephritis [69], puromycin aminonucleoside nephrosis (PAN) [70], adriamycin nephropathy (ADR-N) [71, 72], anti-glomerular basement membrane (GBM) nephritis [73], and diabetic nephropathy [74]; and in glomerular epithelial and endothelial cells cultured in ambient high glucose concentration [75]. Likewise, increased heparanase activity was detected in urine samples from diabetic patients with microalbuminuria [76–78], nondiabetic nephrotic syndrome, chronic kidney diseases (CKD) and kidney transplant patients [76]. Interestingly, neutralization of heparanase activity, using either a sulfated oligosaccharide inhibitor (PI-88) or anti-Hpa antibodies, resulted in reduced proteinuria [79]. Similar findings were reported by Gil and colleagues [60] who demonstrated that Hpa-KO mice failed to develop albuminuria and renal damage in response to streptozotocin-induced diabetes mellitus. Furthermore, albuminuria was attenuated in diabetic mice treated with heparanase inhibitor [60]. These findings are in line with emerging evidence that heparanase is involved in the progression of CKD mainly via activation of profibrotic biological signals including FGF-2 and TGF- β and consequently renal EMT [80].

10 Heparanase in Kidney Transplantation

Since kidney transplantation is associated with renal I/R [81], it is appealing to assume that heparanase/HSPG system is involved in the pathogenesis of delayed graft function (DGF) and chronic allograft nephropathy (CAN). Indirect support for this notion is derived from the observation that heparanase plays a key role in EMT and macrophage polarization following renal I/R damage [20, 21, 82]. Furthermore, Barbas et al. have shown that HS is a novel biomarker for acute cellular rejection, where it is released from the ECM during T-cell infiltration of graft tissue via the enzymatic action of heparanase [83]. A clinical study has shown that plasma HS levels increased significantly in kidney transplant recipients with biopsy-proven acute cellular rejection compared with healthy controls, recipients with stable graft function, and recipients without acute cellular rejection [83]. Similarly, high levels of HS were found in the blood of mice that experienced rejection of cardiac allografts, along with upregulation of heparanase expression in activated T-cells [83]. The expression of Syndecan-1, a transmembrane HS proteoglycan, syndecan-1 sheddases (ADAM17, MMP9) and heparanase was significantly up-regulated after renal transplantation [58, 84]. Increased epithelial syndecan-1 in allografts correlated with low proteinuria and serum creatinine, less interstitial inflammation, less tubular atrophy, and prolonged allograft survival [58]. Celie et al. [59] confirmed the relevance of microvascular basement membrane HSPGs to EC damage in experimental and clinical renal ischemia/reperfusion also in human renal allograft

biopsies. It was found that loss of endothelial expression of the extracellular endosulfatase HSulf-1 may be a likely mechanism underlying the induction of L-selectin and monocyte chemoattractant protein-1 associated with peritubular capillaries in human renal allograft rejection. Also, clinical study revealed a significant correlation between urinary heparanase and plasma levels of this enzyme in transplanted patients. The abundance of heparanase in these patients may lead to kidney damage [77]. Collectively, these findings suggest Hpa/HS as a novel biomarker of acute cellular rejection in solid organ transplantation. Moreover, the association between heparanase, proteinuria and decreased renal function could be translated into new therapeutic options aimed at attenuating chronic renal allograft nephropathy, leading to improved graft survival and patient outcome.

11 Use of Heparanase Related Biomarkers for AKI Detection

In general, biomarkers reporting the activity of heparanase may belong to at least three categories: (1) actual mRNA abundance; (2) protein expression, and (3) abundance of appropriately cleaved HS in body fluids. Studies by Abassi et al. [20] revealed increased abundance of heparanase and its elevated activity in animals with ischemic AKI. With the advent of mass spectroscopy-based detection of glycosaminoglycans (GAGs) in the urine, mainly for the diagnosis of different forms of mucopolysaccharidosis, this technique is gaining popularity in monitoring HS degradation products in diagnosis and prognosis of diverse diseases. In the study by Schmidt and co-authors [85] urinary GAGs (HS, chondroitin sulfate and hyaluronic acid) were measured in patients with sepsis within 24 h of admission to the intensive care unit. These investigators showed that indices of GAG fragmentation and sulfation correlated with the development of AKI. In 100 patients after open heart surgery, high-end mass spectroscopy revealed that cathepsin L, as well as NGAL and cystatin C, are among potential biomarkers of AKI and major adverse kidney events [86]. Reduced urinary excretion of GAGs in women during the first trimester of pregnancy, was measured using electrophoresis on cellulose acetate strips, and it was demonstrated that it has predictive value as an early marker of preeclampsia [87]. Comparative analysis of AKI developing in kidney transplantation from donors after cardiac death vis-à-vis living donors showed that renal microvascular perfusion after the establishment of anastomosis was 42% lower in kidneys that were ischemic prior to the transplantation [88]. This was associated with shedding of the glycocalyx (reduced RBC exclusion zone) and increased production of syndecan-1 and HS.

12 Novel Heparanase Mechanisms-Based Therapies for AKI

In light of the well-established role of Hpa/HSPG in the pathogenesis of various diseases, several inhibitors of heparanase were developed and even reached clinical trials for cancer treatment [89–91]. Among the best-studied heparanase inhibitors, are PG545, SST0001, and PI-88, among other HS mimetics and heparin-derived substances and oligosaccharides [92]. (Hammond and Dredge; Noseda et al., Chhabra and Ferro; in this volume). Also, glycosaminoglycans such as sulodexide inhibit heparanase [93]. The promising results that were obtained so far with these inhibitors in both experimental and clinical studies are not restricted to various malignant diseases, but also to kidney injury. In this regard, it has been demonstrated that heparanase inhibition by either SST0001 or PG545 exerts

nephroprotective effects against I/R –induced experimental AKI as was evident by lowering SCr and BUN levels along with improvement in renal histological features and preventing progression to CKD [20, 21, 94]. In all these studies, the heparanase inhibitors were given as pretreatment, and therefore the efficacy of these inhibitors as post-treatment agents in restoring kidney function and reversing renal remodeling/fibrosis remains to be investigated. Yet, the ability of heparanase inhibitors to abolish FGF-2-induced EMT and TGF- β upregulation in AKI models bear promising potential also in CKD [95] including diabetic nephropathy [60, 96, 97]. Most recently, a pioneer study has demonstrated that aspirin has an inhibitory effect on heparanase [98]. Interestingly, this finding may be responsible for the anti-inflammatory effects of NSAIDs in general and aspirin in particular [99, 100].

13 Summary and Future Perspective

This brief overview on heparanase in AKI incorporates current knowledge on its involvement in inflammation, coagulation, matrix remodeling, and fibrogenesis as related to renal ischemia-reperfusion, nephrotoxicity, sepsis and kidney transplantation complicated by delayed graft function and chronic allograft nephropathy. An intricate pathway of heparanase synthesis, processing and activation are depicted as potentially vulnerable during the course of AKI. Importantly, application of heparanase inhibitors has gained traction in ameliorating AKI. As evident from the above description and a brief history of studies on the role played by heparanase in AKI, it should not come as a surprise that the subject remains in its infancy. Despite significant in-roads into the pathogenesis of AKI and involvement of heparanase, many questions remain open for future investigations. These include, but are not limited to the following:

1. The details on intracellular traffic of pre-pro-heparanase require clarification.
2. The ultimate fate of HS fragments of various sizes in regulating HSPG functions, TLR4 signaling, and trafficking of HSPG awaits resolution.
3. Broader incorporation of diverse nephrotoxic agents into the list of conditions associated with heparanase dysregulation is needed.
4. The optimal timing of pharmacologic intervention to regulate heparanase activity awaits elucidation and justification.

These and other questions will, undoubtedly, illuminate the course of further preclinical and future clinical investigations of heparanase in AKI and other renal diseases.

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Involvement of Heparanase in renal Dysfunction and Histological alterations

Fig. 1.

Comparative characteristics of the course of acute kidney injury (AKI) in mice with heparanase knockout vis-à-vis transgenic mice. Briefly, compared to KO-Hpa, transgenic animals (Tg-Hpa) exhibit enhanced renal activities of pro-inflammatory and epithelia mesenchymal transforming (EMT), leading to decreased Injury healing following I/R, exaggerated destruction of renal architecture, and subsequently more severe functional and excretory deterioration (see text for more details)