Can Plasma Hyaluronan and Hyaluronidase Be Used As Markers of the Endothelial Glycocalyx State in Patients with Kidney Disease?

Hyaluronan (HA) is widely spread in the body and is an important component of the extracellular matrix, including the endothelial glycocalyx (EG). Essential for its vasculoprotective function, HA is involved in vascular permeability and many other processes. In patients with kidney disease, plasma HA is higher than expected, but the extent to which plasma HA and its degrading enzyme hyaluronidase can be used as markers for the state of the EG has not yet been determined.

In the first part of this review, we describe HA synthesis and degradation; we then focus on the involvement of the kidney in the process. In the second part, we summarize the available data on HA and hyaluronidase in patients with kidney failure. Plasma HA is somewhat elevated in kidney failure and predicts for poor survival in dialysis patients. The increased HA levels in kidney failure are probably a result of decreased excretion, but an upregulated turnover cannot be ruled out with certainty in some patients. Hyaluronan might be involved in the regulation of peritoneal transport in PD.

Key words
Endothelium, hyaluronan, hyaluronidase, hemodialysis

Introduction
Hyaluronan (HA) is present in many tissues, including the extracellular matrix and the vascular endothelial glycocalyx (EG). The role of the kidney in the handling of HA and hyaluronidase (Hyal) has been investigated only to a limited extent. Studies on plasma concentrations in patients with kidney failure have not been performed on a large scale, and the extent to which plasma HA reflects the EG is unknown. The objective of the present review is to summarize and discuss the available published data on these matters.

Discussion

HA and Hyal
Hyaluronan is an unsulfated glycosaminoglycan composed of a linear repeat of disaccharide units of D-glucuronic acid and N-acetyl-D-glucosamine. It has a molecular weight in the order of 1000 kDa (HMWHHA), and it is negatively charged, hydrophilic with hydrophobic patches (1), and not bound to a core protein. Hyaluronan is synthesized at the cytosolic site in the cell membrane by HA synthases. A small quantity of lower molecular weight HA (LMWA) is present and can be synthesized either by a specific HA synthase or by degradation of HMWHHA. In contrast to HMWHA, the lower molecular weight fragments are highly angiogenic; they are considered to cause tissue damage—for instance, by induction of cytokines and by increasing synthesis of collagen I (2–4). Hyaluronan is an important constituent of the extracellular matrix. The highest concentrations in humans are present in the umbilical cord and synovial fluid, but data in rabbits suggest that about 8% is present in the kidneys, especially the medulla and papillae (2).

Most of the HA in the vasculature is incorporated into the EG and the extracellular matrix of the underlying tissue (3). Although HA accounts only for 5%–20% of the total glycosaminoglycan content of the endothelial surface layer (5), it is an important...
Hyaluronan and Hyaluronidase in CKD

determinant of the layer’s size, mesh-like structure, and function. Hyaluronan is essential for several vasculoprotective functions of the EG: mechanotransduction of fluid shear stress, NO release, permeability barrier, and adhesion barrier (3,5,6). The essential role of HA within the EG therefore turns it into an attractive potential marker for the state of the EG.

The tissue half-life of HA can range between 0.5 and 3 days and depends mainly on in situ metabolic degradation. The mechanisms for the degradation process include a breaking into smaller fragments by Hyal and also by free oxygen radicals in pathologic situations (2,3). Tissue HMWHA and the LMWHA fragments are taken up into the circulation by the lymphatic system. Lymph nodes extract up to 90% of the quantity transported to the bloodstream. About 85%–90% of the amount reaching the circulation is eliminated in the liver, and 10%, in the kidneys (2).

Six Hyal genes have been identified in mammals. HYAL1 and HYAL2 are the most important ones in humans. The highest levels of HYAL1 messenger RNA are found in the liver, kidney, spleen, and heart. The enzyme resides intracellularly in the lysosomes, has a molecular weight of 57 kDa, and functions optimally at low pH. Hyal-1 cleaves the 1–4 glycosidic bonds of HA into smaller fragments, up to tetrasaccharides (7). Hyal-2 is attached to the outer cell membrane, where it cleaves HA polymers to intermediate-size fragments of about 20 kD. Its optimal function is at a normal extracellular pH (7,8). The assumption is that these fragments are transported intracellularly for further digestion by Hyal-1 (8).

The sources of circulating Hyal are not evident. Plasma concentrations of Hyal-1 can be measured relatively easily, because the assay is done at low pH (9), but how much Hyal-2 can be detected is unclear. Hyal-2 is especially important for the EG, where HA is on the outside of the cell membrane and in direct contact with circulating blood, which makes it likely that Hyal-2 is important in glycocalyx HA turnover.

Plasma HA and Hyal in patients with impaired kidney function

As already stated, plasma concentrations of HA are very low because of elimination in the hepatic sinusoids, lymph nodes, and kidney (2). However, concentrations increase after food ingestion (10), and it would therefore be sensible to take blood samples in a fasting state.

As a consequence of its elimination by the liver, plasma HA has been used to monitor the progression of liver disease (11). At least two studies showed a relationship between the degree of renal function impairment and plasma HA (12,13). The results from the first study suggest that the LMWHA fragments are removed from the circulation by glomerular filtration, but no data on tubular handling of HA are available, although the renal localization of HA occurs especially in the medulla. The finding that the urine of dialysis patients with residual renal function contains only LMWHA might more favor glomerular filtration (14).

As has been shown in a number of studies, impaired HA removal leads to higher HA plasma concentrations in patients with end-stage renal disease, especially those treated with dialysis (14,15).

Little is known about the ratio between LMWHA and HMWHA in the plasma of patients with normal or impaired renal function. In rats, the low molecular weight fraction constituted 26% of total HA in the outer medulla of the kidney (16). In the plasma of hemodialysis patients, LMWHA accounted for one third of the total HA in the 3 patients investigated (14). Plasma of 50 stable peritoneal dialysis (PD) patients contained 15% LMWHA during a follow-up of 6 months (17). Those findings seem to contradict measurements obtained in healthy blood donors and in patients with rheumatoid arthritis or with primary biliary cirrhosis, in whom only HMWHA could be detected (18). The difference supports the contention that, because of impaired renal removal of LMWHA, elevated concentrations of this potentially toxic HA fragment are present in the circulation of patients with end-stage renal disease. The elevated plasma HA concentrations reported in patients with renal failure coincide with elevated levels of various circulating peptides such as adhesion molecules, cytokines, and markers of endothelial dysfunction (13,19). Very high concentrations of HA have been associated with death (15). It has been postulated that the correlations between peptides and HA represent damage to the EG (13). However, the authors putting forward that hypothesis neglected the fact that kidney failure itself leads to increased plasma HA concentrations and that the raised concentrations do not necessarily mean increased biologic effect. In that regard, a relationship between C-reactive protein and plasma HA (19) is absent. The contention that the increased HA concentration in impaired kidney function points to
damage to the EG is therefore at least premature; more probably, the increased concentration is a consequence of decreased renal excretion.

Although hardly any data on the renal handling of Hyal are available, Hyal’s molecular weight of approximately 43 kDa suggests the possibility of removal by glomerular filtration. The finding that urine HA concentrations are about 100 times those in plasma (a urine/plasma ratio similar to that of creatinine) supports the glomerular removal hypothesis (8), but could also be attributable to local release from urinary tract tissue, as appears likely in patients with bladder carcinoma (20).

Combined plasma concentrations of HA and Hyal have been studied in stable end-stage renal disease patients, 23 of them treated with hemodialysis, and 17, with PD (21). Clinically, the patients showed no signs of inflammation. Values of C-reactive protein were also normal in nearly all of them, and 65% were anuric. Plasma HA and Hyal were not different between the PD and hemodialysis patients and were not related to each other; neither were they influenced by plasma C-reactive protein or by the presence or absence of cardiovascular disease. Hyaluronan was evidently related to the duration of dialysis. Plasma HA levels in patients with residual renal function and in healthy controls were similar, but levels were significantly higher in the anuric patients. In contrast, Hyal concentrations were, on average, 31% higher in patients with residual renal function than in healthy controls, but similar in the healthy controls and in the anuric patients. Interestingly, the HA/Hyal ratio was 0.7 in healthy controls and 0.61 in patients with residual renal function, but 1.8 in anuric patients. Those results make it likely that an effect of renal function on plasma HA is present, but limited in the absence of inflammation or cardiovascular disease. Increased Hyal concentrations suggest decreased removal by the kidney, but the normal concentrations in anuria make it likely that Hyal synthesis is impaired in the absence of functioning kidney tissue.

In vitro studies have shown that cultured mesothelial cells and peritoneal fibroblasts synthesize HA (4). Peritoneal deposition of HA in PD patients has especially been described in the submesothelial zone of the parietal peritoneum (4). Concentrations of HA are higher in peritoneal effluent than in plasma, suggesting local production or release (22), and those concentrations increase during acute peritonitis (23). Effluent HA showed no trend over time on PD (24) and was also no predictor for the development of peritoneal sclerosis (25). A switch from conventional to more biocompatible dialysis solution is associated with a decline in effluent HA (26), but no relationships with peritoneal deposition of HA or with peritoneal transport are present.

Maintenance of vascular integrity is an important function of HMWHA (3). Hyaluronan is important in the regulation of water homeostasis, and its negative charge might reduce protein transport through vascular membranes. A study in rats, in which the interstitial mesentery was exposed to either Hyal, chondroitinase, or heparinase, showed an increase in the diffusion coefficient of solutes after exposure to Hyal only (6), suggesting that HA could be involved in the regulation of peritoneal transport. Furthermore, submesothelial HA might affect peritoneal water transport from the circulation to the peritoneal cavity, where the water is removed from the body. In rats with normal kidney function, the addition of HA to a dialysis solution resulted in increased ultrafiltration because of decreased uptake of intraperitoneal fluid into the circulation (27). Such experiments have never been conducted in patients, which makes the value of HA in clinical PD treatment doubtful.

Summary
Hyaluronan is an important component of the extracellular matrix and the vascular EG. The plasma concentrations of HA and, to a lesser extent, of Hyal have been studied extensively in various patient groups, including those with impaired or absent kidney function. Without full determination of the HA and Hyal types, a final interpretation is impossible. However, some facts are evident:

- Plasma HA is somewhat increased in kidney failure, and high values are associated with death.
- It is likely that the increased plasma levels of HA in renal failure are more a sign of decreased excretion than of upregulated turnover; however, a contribution of the latter cannot be excluded.
- Biocompatible PD solutions lower HA concentrations in peritoneal effluent, but the biology of this phenomenon is unknown.
Disclosures
The authors declare that they have no conflicts of interest.

References

Corresponding author:
R.T. Krediet, MD PhD, Nephrology Secretariat, Room F4-215, Academic Medical Centre, Meibergdreef 9, 1105 AZ Amsterdam, Netherlands.
E-mail: r.t.krediet@amc.uva.nl