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Significance of urinary glycosaminoglycans/proteoglycans in the evaluation of type 1 and type 2 diabetes complications

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ABSTRACT

Because of the high incidence of kidney disease in diabetic patients, the early diagnosis of renal impairment is a key point for intervention and management. Although urinary albumin excretion currently represents the accepted standard to assess both diabetic nephropathy and cardiovascular risk, it has some limitations as structural changes in the glomerular basement membrane may occur before the onset of microalbuminuria. It is therefore important to identify urinary markers that may provide greater sensitivity, earlier detection, and greater predictive power for diabetes complications. In this respect, urinary glycosaminoglycans/proteoglycans (GAGs/PGs) have been long associated with several kidney diseases as well as diabetic nephropathies as their levels increase more readily than albuminuria. In particular, heparan sulfate, a key component of the glomerular basement membrane responsible for its charge-dependent permeability, is excreted into urine at higher concentrations during the early kidney remodeling events caused by the altered glucose metabolism in diabetes. Over the past few years, also urinary trypsin inhibitor has been linked to a chronic inflammatory condition in both type 1 and 2 diabetes. The underlying mechanisms of such increase are not completely known since either a systemic inflammatory condition or a more localized early renal impairment could play a role. Nevertheless, the association with other inflammatory markers and a detailed urinary trypsin inhibitor structural characterization in diabetes remain to be elucidated.
This review will discuss a great deal of information on the association between urinary GAGs/PGs and type 1 and 2 diabetes, with particular emphasis on renal involvement, and their potential as markers useful in screening, diagnosis and follow up to be associated with the current standard tests.

1. Introduction

Diabetes mellitus is a huge global health problem affecting more than 380 million people worldwide (International Diabetes Federation, 2013). It represents the fifth-leading cause of mortality and a major risk factor for cardiovascular diseases, such as coronary artery disease, stroke and peripheral vascular disease (hazard ratio of 2.5 for women and 2.4 for men) (Franco, Steyerberg, Hu, Mackenbach, & Nusselder, 2007). Type 1 diabetes mellitus (T1DM) results from the autoimmune destruction of the insulin-producing β cells of Langerhans islets; it is usually diagnosed in children and young adults and represents less than 10% of all cases of diabetes. Type 2 diabetes mellitus (T2DM), or adult-onset diabetes, represents over 90% of cases of diabetes mellitus and is characterized by hyperglycemia caused by insulin resistance. A major issue of T2DM is that it may remain undetected for several years and its diagnosis is often made incidentally, through an abnormal blood or urine glucose test, when vascular complications are already present in most patients (Paneni, Beckman, Creager, & Cosentino, 2013). Currently, it is well known that inflammatory mechanisms, including increased reactive oxygen species (ROS) and prothrombotic factors as well as advanced glycation end-products (AGE) production, play major roles in DM-associated complications that are responsible for elevated indexes of morbidity and mortality.

Since patients with diabetes are at increased risk of microvascular and macrovascular complications (Paneni et al., 2013), the preclinical diagnosis of the state is the key point of the disease management. The renal impairment in diabetes mellitus affects ~40% of type 1 and type 2 diabetic patients. Although chronic kidney disease (CKD) is a common comorbidity condition of T2DM, in the early stages it is often unrecognized, especially in the elderly, and, therefore, untreated (Sarnak et al., 2003). The high incidence of CKD highlights the importance of early diagnosis and treatment for delaying its progression (Meyers, Candrilli, & Kovacs, 2011). Because of the increasing T2DM prevalence worldwide and the serious consequences of this disease on global health, it would be of great value to develop more useful and reliable (and less expensive) diagnostic tests.

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Theoretically, urine represents an excellent substrate for identifying both potential biomarkers for early diagnosis of nephropathy and more effective therapeutic targets or monitoring the progression of renal damage. Urinary albumin excretion (UAE) is currently the "gold standard" for detection or prediction of both diabetic nephropathy and cardiovascular risk, even though in T2DM its predictive power is probably limited to cardiovascular events rather than renal functional impairment. In addition, structural changes in the glomerular basement membrane (GBM) may occur before the onset of microalbuminuria. At the early stages of diabetic nephropathy, characterized by a low urinary excretion of albumin, the charge-dependent glomerular permselectivity seems to be particularly affected (Deckert, Feldt-Rasmussen, Djurup, & Deckert, 1988) suggesting a loss of functional groups in the GBM with a consequent increase in pore size of the renal filtration barrier. This charge-dependent permeability of the GBM is probably due to the presence of anionic constituents, especially heparan sulfate (HS) proteoglycans.

It is therefore important to identify urinary markers that may offer greater sensitivity, earlier detection, and greater predictive power for diabetes complications to overcome limits of UAE tests (Fiseha, 2015).

Since the 80s the association between diabetes complications and glycosaminoglycans/proteoglycans (GAGs/PGs) excretion in urine has been studied allowing the identification of urinary GAGs/PGs, and in particular HS, as potential markers of kidney disease. Accordingly, changes in GAGs/PGs excretion have been reported in association with several kidney pathological conditions (Pugia, Valdes, & Jortani, 2007) such as chronic glomerulonephritis (De Muro et al., 2005, 2007), kidney transplantation (De Muro et al., 2013), and Fabry's disease (Lepedda, Fancellu, et al., 2013; Lepedda, Nieddu, et al., 2013). As their levels increase more readily than UAE during the onset of renal impairment, they may represent useful markers of kidney function to be associated with the common standard tests for early diagnosis and treatment in those patients at high risk.

Over the past few years, also urinary trypsin inhibitor (UTI), a small proteoglycan, has been linked to a chronic inflammatory condition in both type 1 and 2 DM (De Muro et al., 2002, 2006; Lepedda, Fancellu, et al., 2013; Lepedda, Nieddu, et al., 2013).

In this review, the state of the art on the association between urinary GAGs/PGs and the most common forms of diabetes, i.e. type 1 and 2 DM, will be overviewed.

Glycosaminoglycans structure and characterization

Glycosaminoglycans (GAGs) are a group of complex anionic unbranched heteropolysaccharides with both structural and functional roles in many tissues that can be found also in plasma and urine. With the exception of hyaluronan (HA), they are variably sulfated and covalently linked to protein cores forming proteoglycans (PGs). They are composed by repeating disaccharide units containing a hexosamine, either N-acetylgalactosamine (GalNAc) or N-acetylglucosamine (GlcNAc), and a hexuronic acid (glucuronic acid (GlcA) or its carbon-5 epimer iduronic acid (IdoA)) or galactose (in keratan sulfate).

GAGs are very heterogeneous polysaccharides in terms of type of repeating disaccharide unit, relative molecular mass, charge density, degree and pattern of sulfation, degree of epimerization and physicochemical properties. Numerous biological functions of PGs mainly depend on specific structural characteristics of their GAG chains (D.H. Vynios, Karamanos, & Tsiganos, 2002).

Six main classes of GAGs have been described: hyaluronan or hyaluronic acid (HA), chondroitin sulfate (CS) and dermatan sulfate (DS), keratan sulfate (KS), heparan sulfate (HS) and heparin (HE) (Fig. 1).

Usually, GAGs are extracted from connective tissues or plasma by proteolytic treatment with papain or proteinase k (this step is not required with urine samples) and purified by anion exchange chromatography (De Muro et al., 2002; Naso et al., 2010). Then, GAGs are evaluated quantitatively by colorimetric assays. In this respect, Carbazole and Dimethylmethylene Blue Assays are the most used methods. The first one allows to assess hexuronic acid content in a sample by the reaction with carbazole in sulfuric acid (Bitter & Muir, 1962), whereas the latter is based on the metachromatic shift which occurs when the dye is complexed with sulfated glycosaminoglycans, regardless the GAG species (Farndale, Sayers, & Barrett, 1982). The evaluation of GAGs composition is rather performed by electrophoretic analyses, mainly on cellulose acetate or agarose gel matrices (Volpi & Maccari, 2006). Furthermore, a structural characterization of GAGs could be performed mainly by fluorophore-assisted carbohydrate electrophoresis, high-performance liquid chromatography, or capillary electrophoresis after GAG depolymerization with specific lyases (Vynios et al., 2002).

2. Plasma and urinary GAGs/PGs

The main circulating GAG is a low sulfated chondroitin sulfate linked to a protein core to form the small proteoglycan bikunin (Imanari et al., 1992). Bikunin consists of a small polypeptide of 147 amino acid residues, which carries an N-linked oligosaccharide at Asn^{45} and a O-linked low-charge chondroitin sulfate chain (Fries & Blom, 2000; Zinellu et al., 2007, 2012), at Ser^{10}, with Ser-proteinase inhibitory activity. Synthesized by hepatocytes, bikunin circulates primarily as a subunit of the inter-α-inhibitor (IαI) family molecules, covalently linked, via the CS chain, to one or two polypeptides (heavy chains, HCs) (Zhuo, Hascall, & Kimata, 2004). After an inflammatory stimulus, IαI leaves the circulation and, in extravascular sites, the heavy chains are transferred from the CS chain to the locally synthesized hyaluronan to form the serum-derived hyaluronan associated protein–hyaluronan complex (SHAP-HA). This complex plays important roles in stabilizing extracellular matrices and it is often associated with inflammatory conditions (Zhuo et al., 2004). When bound to IαI, bikunin lacks some of its known activities, and there is evidence that its release, by partial proteolytic degradation, may function as a regulatory mechanism. Beside its proteinase inhibitory activities, for example, toward plasmin during tumor cell invasion and metastasis (Kobayashi H, Gotoh J, et al. 1995; Kobayashi, Shinohara H et al., 1996; Kobayashi H et al. 2003), bikunin plays additional roles, such as inhibition of interleukin (IL)-8 gene expression induced by lipopolysaccharide (Maehara et al., 1995), smooth muscle contraction (Kanayama et al., 1995, 1998), neutrophil release of elastase (Hiyama, Takeda, Kotake, Morisaki, & Fukushima, 1997), mast cell release of histamine (Kobayashi, Shibata, Fujie, & Terao, 1998), suppression of immune cells (Cowan et al., 1996; Kato, Nagao, & Kurosawa, 1995; Nakatani & Takeshita, 1999), and urolithiasis (Atmani, Glenton, & Khan, 1999, Khan & Kok, 2004), as well as stabilization of lysosomal membranes (Kato et al., 1998; Nakakuki et al., 1996).

Free bikunin is rapidly cleared from circulation by both tissue uptake and renal excretion (Kaczmarczyk, Blom, Alston-Smith, Sjöquist, & Fries, 2005) and it is found in urine as the urinary trypsin inhibitor (UTI) also referred to as LSC-PG (low-sulfated chondroitin sulfate proteoglycan). UTI levels are usually lower than 5 μg/ml in healthy individuals, but they increase up to tenfold in both acute and chronic inflammatory diseases (Jortani et al., 2004; Lin et al., 2004; Matsuzaki et al., 2005; Mizon et al., 2002; Tsui et al., 2010). It has been reported that also the chondroitin sulfate chain of bikunin can be modified during inflammatory conditions in terms of both sulfation degree and chain length (Capon, Mizon, Lemoine, Rodié-Talbère, & Mizon, 2003). According to a plethora of papers, bikunin can be considered a positive acute phase protein (Pugia & Lott, 2005).

In urine, GAGs consist mainly of HS, CS, and, in negligible quantity, DS (Esko, Kimata, & Lindahl, 2009). Besides UTI, also UTI derivatives, i.e. low-sulfated chondroitin sulfate (LSC) and slow-migrating LSC (SM-LSC), can be found (De Muro et al., 2007). Urinary HS derives mainly from the turnover of heparan sulfate proteoglycan (HS-PG), the main anionic component of the GBM responsible for its charge selectivity. A quote of urinary GAGs/PGs is of plasma origin deriving from glomerular filtration of plasma bikunin.

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