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REVIEW

The 'sweet' and 'bitter' involvement of glycosaminoglycans in lung diseases: pharmacotherapeutic relevance

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The extracellular matrix (ECM) plays a significant role in the structure and function of the lung. The ECM is a three-dimensional fibre mesh, comprised of various interconnected and intercalated macromolecules, among which are the glycosaminoglycans (GAG). GAG are long, linear and highly charged, heterogeneous polysaccharides that are composed of a variable number of repeating disaccharide units (macromolecular sugars) and most of them, as their name implies, have a sweet taste. In the lung, GAG support the structure of the interstitium, the subepithelial tissue and the bronchial walls, and are secreted in the airway secretions. Besides maintaining lung tissue structure, GAG also play an important role in lung function as they regulate hydration and water homeostasis, modulate the inflammatory response and influence lung tissue repair and remodelling. However, depending on their size and/or degree of sulphation, and their immobilization or solubilization in the ECM, specific GAG in the lung either live up to their sweet taste/name, supporting normal lung physiology, or they are associated to 'bitter' effects, related to lung pathology. The present review discusses the biological role of GAG in the lung as well as the involvement of these molecules in various respiratory diseases. Given the great structural diversity of GAG, understanding the changes in GAG expression that occur in lung diseases may lead to novel targets for pharmacological intervention in order to prevent and/or to treat a range of lung diseases.
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Abbreviations: COPD, chronic obstructive pulmonary disease; ECM, extracellular matrix; GAG, glycosaminoglycans; HA, hyaluronic acid; HAS, hyaluronic acid synthase; HYAL, hyaluronidase; IL, interleukin; IPAH, idiopathic pulmonary arterial hypertension; PASMC, pulmonary arterial smooth muscle cells; RHAMM, receptor for HA-mediated motility; TGF, transforming growth factor; TNF, tumour necrosis factor; VEGF, vascular endothelium-derived growth factor

Introduction

The structure of the extracellular matrix (ECM) in the lung plays several important roles in lung function: (i) it offers a low resistant pathway, allowing effective exchange of gases; (ii) it provides tensile and compressive strength and elasticity, with a strong and expandable framework that supports the fragile alveolar-capillary intersection; (iii) it controls the behaviour of cells by binding of growth factors and by interaction with cell surface receptors; and (iv) it acts as a buffer against retention of water (Negrini *et al.*, 2000; Suki *et al.*, 2005). The alveolar wall is composed of an epithelial cell layer and its basement membrane, the capillary basement membrane and endothelial cells, and the ECM, which is a thin layer of interstitial space lying between the capillary endothelium and the alveolar epithelium (West and Mathieu-Costello, 1999). In some areas, the two basement membranes are physically fused to reduce the diffusion distance. In the segments where the two basement membranes are not fused, the interstitium is composed of cells, a macromolecular fibrous component and the fluid phase of the ECM. In these parts, the ECM functions as a three-dimensional mechanical scaffold characterized by a fibrous mesh consisting mainly of collagen type I and III, providing tensile strength, and elastin (Suki *et al.*, 2005). The three-dimensional fibre mesh is filled mainly with glycosaminoglycans (GAG) (macromolecular

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sugars), which are the major components of the nonfibrillar compartment of the interstitium (Negrini *et al.*, 2000). The majority of GAG, as their name implies, have a sweet taste (glycos in Greek means sweet).

Although many studies have described the role of proteins, proteoglycans, growth factors and other type of molecules in a wide range of pulmonary diseases (Turino and Cantor, 2003; Bai *et al.*, 2005), the actions of GAG in lung pathophysiology are much less well understood. GAG have overall attracted less attention by researchers, due to the fact that they are abundant in almost all tissues, they have very high molecular mass, immense size versatility and they are not directly encoded by a single gene. However, the improvement of techniques for the isolation, purification and characterization of GAG, the discovery of specific GAG synthases and GAG-degrading enzymes and, most importantly, the recent key technologies regarding carbohydrate sequencing and the synthesis of defined oligosaccharides have contributed to progress in glycomics research. Synthetic tools and high-throughput experiments, such as carbohydrate arrays, are beginning to affect biological research. These techniques are now being applied to the development of carbohydrate-based diagnostics, vaccines and therapeutics (Peter *et al.*, 2007). With respect to lung structure and function, there is an accumulation of evidence for the involvement of GAG in lung physiology and pathology. The comprehensive study of GAG will improve our knowledge on the development of lung diseases, may allow early diagnosis of ECM alterations and lung remodelling processes, and may promote development of novel targets for pharmacological interventions.

Glycosaminoglycans

GAG represent major components of the ECM that undergo significant alterations in content, synthesis and distribution during neonatal organ growth (Schmid *et al.*, 1982), acute injury (Cantor *et al.*, 1980), degenerative diseases or age-related tissue modifications (Kumar *et al.*, 2005; Vitanzo and Sennett, 2006; Blewis *et al.*, 2007; Ito *et al.*, 2007; Wang *et al.*, 2007).

With respect to their structure, GAG are long, linear and heterogeneous polysaccharides, which consist of repeating disaccharide units, with sequences that vary in the basic composition of the disaccharide unit, type of linkage and degree of acetylation and sulphation. The disaccharide units are galactose, N-acetylglucosamine, N-acetylgalactosamine, glucuronic acid or iduronic acid (Figure 1). The chain length of GAG can range from several hundred to several thousand disaccharide units, and thus their molecular mass may vary over three orders of magnitude, implying that the polymer chains have great variability in size and structure (Scott, 1992). There are two main types of GAG: the non-sulphated GAG hyaluronic acid (HA) and the sulphated GAG (heparan sulphate and heparin, chondroitin sulphate, dermatan sulphate and keratan sulphate). With the exception of HA, GAG are usually covalently attached to a protein core (Figure 1), forming overall structures referred to as proteoglycans (Johnson *et al.*, 2005).

GAG are either an integral part of the ECM or they are located directly on the cellular membrane, where they can function as protein receptors or activators. The turnover of these local GAG and their composition is essential for organ function and homeostasis. In order to understand the biological function of GAG, one must be aware that GAG molecules are not encoded by a single gene, as already mentioned, but are synthesized by specific synthases, which are regulated by tissue or cell type local microenvironments. Moreover, GAG are not synthesized by the action of a single synthase, but result from the as yet poorly understood interactions of several independently regulated synthases (Funderburgh, 2002; Itano and Kimata, 2002; Kusche-Gullberg and Kjellen,

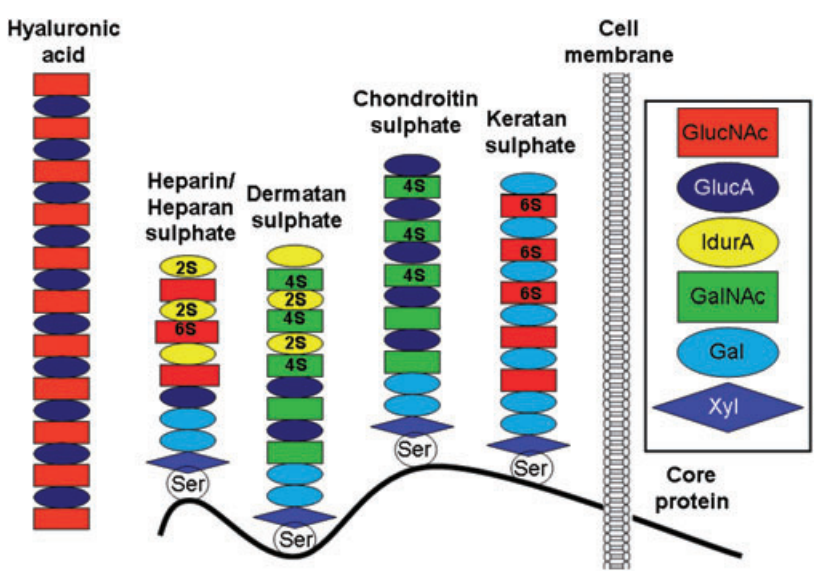

Figure 1 Schematic structure of glycosaminoglycans and proteoglycans. In the extracellular matrix, heparin, heparan sulphate, dermatan sulphate, chondroitin sulphate and keratan sulphate are connected to a transmembrane protein core via a serine residue. Hyaluronic acid is not linked to a protein core. GlucNAc, N-acetylglucosamine; GlucA, D-glucuronic acid; IdurA, L-iduronic acid; GalNAc, N-acetylgalactosamine; Gal, galactose; Xyl, xylose; Ser, serine; 2S, 4S, 6S, position of sulphate residue on the sugar moiety.

2003). Furthermore, there is evidence that during the assembly of GAG the addition of sulphate residues to the GAG chain determines their function. For example, it has been established that the anticoagulant activity of heparin depends on specific binding to antithrombin and that the antithrombin binding site on heparin is a pentasaccharide with specific modifications by sulphation (Sugahara, 1992).

Biological function of GAG in the lung

Hyaluronic acid

HA is the most abundant non-sulphated GAG in the lung ECM. It differs from the other GAG because it is spun out from the cell membrane, rather than being secreted through the Golgi apparatus. HA is a linear polysaccharide composed of up to 10 000 disaccharides, constituted by a uronic acid residue covalently linked to (1-b-3) N-acetyl-D-glucosamine, with a flexible and coiled configuration. Thus, HA has a high molecular mass (up to 10^7 Da), which is considerably larger than other GAG. In solution, the molecular chain behaves as an extended random coil, with a diameter of approximately 500 nm (Laurent, 1970). At concentrations as low as 0.1%, the molecular chains were entangled, resulting in an extremely high, shear-dependent viscosity. These properties enable HA to regulate water balance, osmotic pressure and flow resistance, to interact with proteins, and also to act as a sieve, lubricant, and to stabilize structures by virtue of its electrostatic interactions (Laurent, 1970). It is a ubiquitous molecule of the connective tissue matrix and is necessary for its assembly and stabilization (Gerdin and Hallgren, 1997). A unique characteristic of HA that relates to its various functions is its high anion charge, which attracts many water molecules offering tissue hydration (Turino and Cantor, 2003). Therefore, accumulation of HA in the interstitial tissue may immobilize water and regulate the water content in the interstitium (Gerdin and Hallgren, 1997). HA is also involved in several lung functions, such as tissue repair, which is achieved via regulation of HA synthase 2 and hyaluronidase 2 in response to mediators released during the healing process after lung injury, thus leading to the fibroproliferative response (Li *et al.*, 2000b). Furthermore, HA enhances cellular host defence mechanism by stimulating the activity of blood neutrophils, such as phagocytosis and free oxygen radical formation and migration (Turino and Cantor, 2003). In this respect, it has been shown that subcutaneous administration of HA reduced the number of bacterial infections in patients with an increased susceptibility to such infections. Patients with chronic bronchitis and recurrent acute exacerbation of their disease, who were treated with HA, had significantly fewer acute exacerbations than did placebo-treated patients (Venge *et al.*, 1996). In addition, HA seems to protect against proteolytic enzymes as it has been reported that aerosol preparation of streptococcal HA prevents experimental emphysema induced by intratracheal administration of porcine pancreatic elastase (Cantor *et al.*, 2000). In the pulmonary alveolus, HA interacts with surfactant phospholipids, contributing to the stability of the surfactant layer (Lu *et al.*, 2005).

In the lungs, the content of HA is 15–150 $mg \cdot g^{-1}$ dry weight (species specificity), which is mainly localized in the peribronchial and interalveolar/perialveolar tissue (Petrigni and Allegra, 2006). The quantity of HA in human lung secretions was found to be approximately 66 $ng \cdot mL^{-1}$ with values ranging from 34 to 423 $ng \cdot mL^{-1}$ (Dentener *et al.*, 2005).

Polymerization of HA occurs by the action of one or more of the three HA synthases (termed HAS 1, HAS 2 and HAS 3) (Itano *et al.*, 1999), through the joining of the glycosidic residues to the reducing chain extremity, and is extruded while still elongating into the ECM (Stern, 2003). HAS isoforms have distinct catalytic rates, resulting to HA products of different molecular mass (Itano and Kimata, 2002). HAS 1 and HAS 2 synthesize relatively long stretches ($2–4 \times 10^6$ Da). HAS 1 is the least active, while HAS 2 is more catalytically active, and therefore might be the main enzyme responsible for increased synthesis found in stress-induced conditions and wound healing (Stern, 2005). HAS 3 is the most active isoform, it polymerizes short stretches of disaccharide chains ($0.4–2.5 \times 10^5$ Da) (Itano *et al.*, 1999; Weigel *et al.*, 1997) and may be involved in activation of signal transduction (Stern, 2005). HA is metabolized by hyaluronidases (HYAL), mainly by HYAL 1 and HYAL 2, present in various tissues, including the lung (Dentener *et al.*, 2005).

Two cell-associated receptors, CD44 and RHAMM (receptor for HA-mediated motility), mediate the biological effects of HA (Slevin *et al.*, 2007). CD44 and RHAMM are abundant in different cell types. For example, CD44 is expressed in fibroblasts, epithelial and smooth muscle cells and immune cells, such as neutrophils, macrophages and lymphocytes (Sherman *et al.*, 1994), while RHAMM is expressed in cells following injury (Savani *et al.*, 1995), in tumours and in some mutant active Ras-transformed cell lines (Li *et al.*, 2000a; Ahrens *et al.*, 2001). CD44 is a type I integral cell surface membrane glycoprotein that participates in cell–cell and cell–matrix adhesion (Naor *et al.*, 2002). HA binds to the extracellular domain of CD44 and subsequently the cytoplasmic tail of CD44 activates intracellular signalling pathways that regulate the interactions of signalling complexes with actin cytoskeleton (Toole, 2004). The interaction of HA with CD44 regulates leucocyte rolling and activation, as well as tumour metastasis. Furthermore, CD44-dependent clearance of HA fragments is crucial in resolving lung inflammation in the bleomycin model of lung injury (Teder *et al.*, 2002), demonstrating an essential role for CD44 in the resolution phase of inflammation.

HA also binds to RHAMM, which controls the effect of HA on cell migration, proliferation and motility, apparently via RHAMM interaction with the cytoskeleton (Savani *et al.*, 1995; Turley *et al.*, 2002). Cell surface RHAMM occurs in cell lamellae and podosomes (Turley, 1980) and exogenous HA has been reported to promote spreading of cell lamellae and turnover of focal adhesions in fibroblasts transfected with oncogenic Ras that express cell surface RHAMM (Hall *et al.*, 1994). Agonist RHAMM antibodies mimic, whereas blocking RHAMM antibodies inhibit, this effect (Bourguignon *et al.*, 2001). The rapid formation and then disassembly of focal adhesions precede the HA-induced increase in cell motility (Hall *et al.*, 1994). It has been reported that these effects are possibly mediated through the integrin fibronectin receptors, $\alpha_4\beta_1$ or $\alpha_5\beta_1$ (Gares and Pilarski, 2000). Evidence of increased focal adhesion turnover following addition of HA to migrating cells highlights the dynamic role of the pericellular matrix

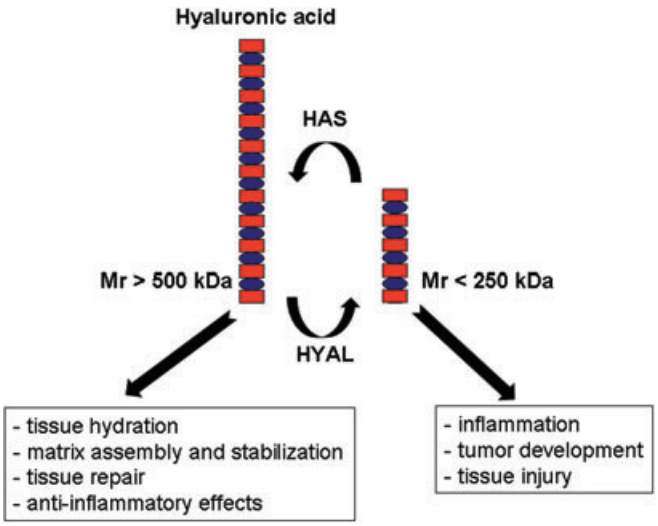

Figure 2 The biological functions of hyaluronic acid of low and high molecular mass in human cells and tissues. HAS, hyaluronic acid synthases; HYAL, hyaluronidases.

(Hall *et al.*, 1994). Rapid uptake of HA and translocation to the nucleus, with signalling mediated through RHAMM, was associated with increased motility. With respect to the effects of HA on proliferation via HA-RHAMM interaction, it has been reported that intracellular RHAMM associates with the actin cytoskeleton and the mitotic spindle microtubules (Assmann *et al.*, 1999). HA is also internalized in premitotic cells and co-distributes around the mitotic spindle with microtubule-associated RHAMM (Evanko *et al.*, 2004). Microtubules are compression bearing elements of the cytoskeleton that target focal adhesions for dissolution (Kaverina *et al.*, 1999). This raises the possibility that HA internalized from the pericellular matrix may also regulate or facilitate events involving microtubules during cell shape changes. The ability of RHAMM to form coiled coils as well as its partial homology with proteins such as D-CLIP (Akhmanova *et al.*, 2001) that link microtubules and actin filaments suggest that intracellular RHAMM proteins may connect actin and microtubule cytoskeleton (Assmann *et al.*, 2001). As RHAMM has been associated with several kinases, it is likely that intracellular RHAMM proteins act as adapter molecules (Bustelo, 2000) that connect the cytoskeleton to signalling complexes. Transcriptional profiling of the cell cycle progression reveals enhanced expression of both RHAMM and HAS 2 at the G_2M boundary (Cho *et al.*, 2001). These results attribute a possible role of intracellular HA/RHAMM interactions in proliferation.

Several studies have demonstrated that the biological effects of HA appear to vary depending on its average molecular mass (Uchiyama *et al.*, 1990; McKee *et al.*, 1996; Ohkawara *et al.*, 2000; Horton *et al.*, 2002; Tammi *et al.*, 2002; Turino and Cantor, 2003; Mascarenhas *et al.*, 2004; Bai *et al.*, 2005) (Figure 2). In physiological conditions, HA has a high average molecular mass, in excess of 10^6 Da. However, following tissue injury, degradation of HA may occur intracellularly (Rodén *et al.*, 1989) or extracellularly (Lin *et al.*, 1994) leading to the formation of HA fragments of lower molecular mass, of about 0.25×10^6 Da, which can induce the expression of inflammatory genes (Tammi *et al.*, 2002). Low-molecular-weight fragments can stimulate activated macrophages to express RNAs of numerous chemokines and cytokines (McKee *et al.*, 1996; Horton *et al.*, 2002), whereas fragments of higher molecular weight have an opposite effect and suppressed chemokine expression (Turino and Cantor, 2003). It has been shown that HA of low molecular mass stimulated the maturation of dentritic cells and the synthesis of proinflammatory interleukin (IL)-1β, IL-12, and tumour necrosis factor (TNF)-α (Taylor *et al.*, 2004). The latter effect seems to be restricted to an interaction of HA fragments with the toll-like receptor 4 (Termeer *et al.*, 2002; Taylor *et al.*, 2004). HA of low molecular mass has also been shown to stimulate macrophages to produce important mediators of tissue injury and repair, such as inducible nitric oxide synthase, plasminogen activator inhibitor-1, macrophage inflammatory protein-1α and -1β, TNF-α and macrophage metalloelastase (McKee *et al.*, 1996; 1997; Horton *et al.*, 1998a; 1998b; 1999; 2000). HA fragments can synergize with interferon-γ to induce the antifibrotic chemokines monokine induced by interferon-γ and interferon-inducible protein-10 (Horton *et al.*, 1998b). The transcriptional mechanism involved in mediating the synergy between HA and interferon-γ on monokine induced by interferon-γ gene expression was identified to be activation of nuclear factor (NF)-kappaB (Horton *et al.*, 2002). There are no available data regarding the molecular mechanisms via which HA of high molecular mass suppress chemokine expression.

Furthermore, it has also been shown that fragmented HA, which induces inflammatory gene expression *in vitro*, is in the same size range as HA that accumulates under inflammatory conditions *in vivo* (McKee *et al.*, 1996). It seems that under conditions of inflammation, tumour development or tissue injury, HYAL activity or oxidation generate HA fragments that subsequently can initiate gene transcription, influencing cell proliferation and migration (Uchiyama *et al.*, 1990).

Heparan sulphate

Heparan sulphate is the most abundant sulphated GAG within the lung parenchyma with a size ranging from 0.005 to 0.07×10^6 Da. It is expressed on virtually every cell in the body and comprises 50–90% of the total endothelial proteoglycans (Handel *et al.*, 2005). Heparan sulphate has the most variable structure, mainly due to variations in the sulphation patterns of its chains. It is initially produced in a cell surface-bound form, but it can also be secreted as a soluble GAG.

It has been shown that heparan sulphate interacts with various proteins affecting the topography, half-life and bioactivity of the protein. Furthermore, heparan sulphate is involved in a variety of biological processes, including cell–matrix interactions and activation of chemokines, enzymes and growth factors (Nader *et al.*, 1987; Whitelock and Iozzo, 2005). In this context, the interaction between fibroblast growth factors (FGFs) and their tyrosine kinase receptors depends on the sequence of the heparan sulphate chain (Handel *et al.*, 2005). Heparan sulphate plays a critical role in FGF signalling by facilitating and/or stabilizing the formation of FGF–FGF receptor complexes and enhancing FGF oligomerization (Raman *et al.*, 2005). In addition, heparan sulphate binds FGF in the ECM, storing it in an inactive form until needed, thereby allowing rapid response to stimuli (Aviezer *et al.*, 1999).

Heparan sulphate interacts with cytokines such as IL-5, IL-6, IL-8, IL-10, TNF-α and platelet factor-4 (Lipscombe *et al.*, 1998; Mummery and Rider, 2000; Menart *et al.*, 2002). Binding of heparan sulphate to cytokines is due to noncovalent and reversible interactions; however, the precise mechanism of these interactions at the molecular level is not known. This binding is likely to retain cytokines, which are otherwise small, readily diffusible proteins, close to their sites of release in the tissues, thus favouring paracrine rather than endocrine activity (Mummery and Rider, 2000). Because many cytokines are highly pleiotropic, interaction with heparan sulphate in the vicinity of their secretion represents a mechanism of regulation, as the cytokine would be mostly available only to neighbouring target cells. However, some release of the cytokine into the circulatory system could still occur, either because the binding interactions are noncovalent and reversible, or because of the fragmentation of the proteoglycan macromolecule involved by the action of proteases or heparinases. In the latter case, the cytokine could remain bound to heparan sulphate-peptide fragments or heparan sulphate oligosaccharides. Beyond such a general role in compartmentalizing cytokines within the tissues, the binding of cytokines to heparan sulphate has been shown to have particular roles for individual cytokines (Mummery and Rider, 2000). Thus, the interaction of heparan sulphate with IL-8 promotes the activity of the cytokine, whereas in the case of platelet factor-4, the interaction inhibits the activity. The binding of FGF-2 to heparan sulphate has been shown to have an essential role in the signalling pathway of the growth factor, because it is a prerequisite for subsequent engagement to the high-affinity cell surface FGF-2 receptor (Rapraeger *et al.*, 1991; Yayon *et al.*, 1991). These examples illustrate that the binding of a cytokine to heparan sulphate may have major consequences on cytokine activity, and that these may be quite different from one cytokine to another.

Heparan sulphate has also been shown to interact with various ECM proteins, including fibronectin, laminin, thrombospondin, collagen types I, II, IV, V, VI, XIII and XVIII, and endostatin. The binding of endostatin by heparan sulphate is important for its antiangiogenic function (Kreuger *et al.*, 2002). The interaction between heparan sulphate and laminin could be important in determining the integrity of basement membranes (Parthasarathy, 1998). The interaction between heparan sulphate and collagen type V plays an important role in the modulation of cell adhesion to the substratum (LeBaron *et al.*, 1989).

Heparin

Heparin is an over-sulphated variant of heparan sulphate, commonly used as an anticoagulant drug (Whitelock and Iozzo, 2005). Heparin has a molecular mass ranging from 0.006–0.05 $\times$ 10^6 Da and shares many structural and functional activities with heparan sulphate. The lung is a rich source of heparin as the lung is abundant in mast cells, which are responsible for the bulk synthesis of heparin (Poole, 1986). Mast cell heparin resides in secretory granules, where most of the GAG chains are linked to a core protein (serglycin), forming macromolecular proteoglycans that are much larger than commercial heparin.

Endothelial cells may also produce a highly sulphated heparin that is capable of binding antithrombin III directly on the vascular wall, preventing adherence of platelets and clotting factors to the vessel wall (Merry *et al.*, 1999). In respect to vessel integrity and tissue remodelling, as well as tumour development, the interaction of heparins with specific isoforms of the vascular endothelium-derived growth factor (VEGF) and its receptors is of interest. It has been shown that heparin binds and activates VEGF-A145 and -A165, but not -A121; it binds to VEGF-B167, but not to -B186; it binds to VEGF-E, -F1 and F2, but not to VEGF-C or -D (Soker *et al.*, 1994). The capacity of heparin to bind specific VEGF isoforms depends on the number of sulphate groups, and the localization of VEGF in the tissue is assumed to be directed by heparins (Springer *et al.*, 2007). The effect of heparins on the function of VEGF is highly controversial. In some studies, heparins enhanced the VEGF function, which is of specific interest as heparin is used in patients with neoplasia (Pyda *et al.*, 2006), while in other conditions heparins counteracted VEGF actions (Moy *et al.*, 2006; Wijelath *et al.*, 2006).

Many studies have shown that heparin plays a beneficial role in the regulation of the inflammatory response (Page, 1997). In this context it has been shown that heparin can inhibit the influx of neutrophils into certain tissues and inhibit T cell trafficking, partly by an inhibitory effect on the heparinase enzyme secreted by T cells (Tyrell *et al.*, 1995). Furthermore, heparin has been shown to be released from human lung mast cells in response to allergen exposure, and increased levels of a heparin-like substance have been reported in the plasma of asthmatic individuals (Green *et al.*, 1993). Heparin can also inhibit allergen-induced eosinophil infiltration into the airways of experimental animals (Ahmed *et al.*, 1993). After the inflammatory cells have passed through the lung tissue, it is recognized that there is a number of stages involved, including adhesion to the vascular endothelium, diapedesis across the endothelial cells and chemotaxis within tissues. It is clear that heparin can inhibit all stages of cell migration, including the carbohydrate–selectin interactions between endothelial cells and leucocytes, the presentation of specific chemoattractants to activated leucocytes and leucocyte trafficking. Endothelial heparan sulphate has also been reported to act as a ligand for L-selectin during neutrophil rolling (Wang *et al.*, 2005). Although the above mechanisms underlie the effect of heparin on neutrophil migration, the ability of heparin to interfere with eosinophil adherence is less well understood. Nonetheless, heparin is able to inhibit the actions of several important eosinophil chemoattractants, such as platelet factor-4 (Tyrell *et al.*, 1995).

Although the precise mechanism of the anti-inflammatory effects of heparin has not been clarified, it has been suggested that inhibition of the interaction between pro-inflammatory cytokines and membrane-associated GAG may provide a mechanism for inducing clinically useful immunosuppression. Whereas immobilized heparin is essential for the biological activity of chemokines, soluble heparin has been shown to inhibit the biological effects of chemokines (Kuschert *et al.*, 1999). This may be explained as follows: chemokines can form complexes with either cell surface or soluble GAG and these interactions lead to different functions. Immune modulators, such as chemokines and growth factors,

exert their biological activity through high-affinity interactions with cell surface receptors, thereby activating specific signalling pathways. Many of these molecules also participate in low-affinity interactions with another class of molecules, referred to as proteoglycans, which consist of a protein core to which GAG chains are attached. Certain chemokines require interactions with GAG for their *in vivo* function (Handel *et al.*, 2005). The GAG interaction is thought to provide a mechanism for retaining chemokines on cell surfaces, facilitating the formation of chemokine gradients. These gradients serve as directional cues to guide the migration of the appropriate cells in the context of their inflammatory, developmental and homeostatic functions. In contrast, soluble GAG chemokines complexes are unable to bind the receptor, resulting in a block of the biological activity. It is therefore likely that the anti-inflammatory effects of heparin are mediated, at least in part, by interference with the chemokine system (Handel *et al.*, 2005).

Chondroitin sulphate

Chondroitin sulphate is a key regulator for ECM protein activation and degradation. The function of chondroitin sulphate depends on the location of its sulphate groups, which determines its structure and binding characteristics. It has been shown that chondroitin sulphate activates ECM-degrading enzymes, such as matrix metalloproteases (MMP). MMP constitute a heterogeneous family of 28 proteolytic enzymes (Visse and Nagase, 2003; Tu *et al.*, 2008). Their common features include the presence of a Zn atom in their active site and their involvement, after activation, in basement membrane remodelling, wound contraction, neovascularization, tissue repair and generally ECM homeostasis (Visse and Nagase, 2003; Tu *et al.*, 2008). MMP are also involved in lung disorders (Greenlee *et al.*, 2007) and hypoxia-associated lung diseases (Karakiulakis *et al.*, 2007). The MMP gene family encodes for several proenzymes (proMMP), with common and distinctive structural and functional properties, classified as: (i) collagenases 1–4 (MMP-1, -8, -13 and -18 respectively); (ii) gelatinases A and B (MMP-2 and -9 respectively); (iii) stromelysins 1–3 (MMP-3, -10 and -11 respectively); (iv) matrilysins 1 and 2 (MMP-7 and -26 respectively); (v) membrane types 1–6 (MT-MMP: MMP-14, -15, -16, -17, -24 and -25 respectively); and (vi) various others (MMP-12, -19, -20, -21, -23, -27 and -28) (Visse and Nagase, 2003). ADAMTS (a disintegrin and metalloproteinase with thrombospondin motifs) -4 and -7B have also been implicated in a number of connective tissue pathologies (Jones, 2006). The activity of MMP is tightly regulated during proMMP expression (Stetler-Stevenson *et al.*, 1993) and by proteolytic activation of secreted latent MMP (Cao *et al.*, 1995). Most MMP are not constitutively expressed *in vivo* but can be rapidly induced in response to signals, such as growth factors, cytokines, phorbol esters and cell–cell or cell–ECM interactions (Tu *et al.*, 2008). MMP, both in latent and activated form, are also regulated by the formation of stoichiometric 1:1 complexes with specific inhibitors, known as tissue inhibitors of MMP (Stetler-Stevenson *et al.*, 1993). Chondroitin sulphate has been reported to be involved in the degradation of aggrecan, which is a chondroitin sulphate-rich proteoglycan, by MMP-2 (Rodriguez *et al.*, 2006; Iida *et al.*, 2007), MMP-13 (Miwa *et al.*, 2006), ADAMTS4 (Miwa *et al.*, 2006) and ADAMTS7B (Somerville *et al.*, 2004). MMP-2 is activated by direct binding of chondroitin sulphate to the C-domain of proMMP-2, which is essential to present the inactive enzyme to its activator, the MT-MMP-3 (Iida *et al.*, 2007).

Chondroitin sulphate has also been reported to have anti-inflammatory properties in osteoarthritis, preventing joint space narrowing and reducing joint swelling and effusion. These effects are produced by reduction of NF-kappaB nuclear translocation, probably by diminishing extracellular signal-regulated kinase, p38 mitogen-activated protein kinase and c-Jun N-terminal kinase activation (Iovu *et al.*, 2008).

Dermatan sulphate

Dermatan sulphate binds to a variety of proteoglycans, affects the function of growth factors, modifies the action of the heparin cofactors (Sugahara *et al.*, 2003; Kinsella *et al.*, 2004; Villena and Brandan, 2004) and influences proliferation in a cell type-specific manner. Dermatan sulphate also occurs as a circulating GAG in the blood and binds to several proteins that are part of the coagulation cascade, including activated protein C, heparin cofactor II, fibrinogen, fibronectin, apolipoprotein B, kininogen, trypsin inhibitor A and factor H. All these proteins are sensitive to serine protease. Dermatan sulphate can activate serine protease with similar capacity to heparan sulphate, but the mechanism of action of dermatan sulphate activation of these proteases is unclear (Saito and Munakata, 2007). Binding of dermatan sulphate to activated protein C and heparin cofactor II accounts for its antithrombotic activity (Du *et al.*, 2007).

Furthermore, dermatan sulphate functions as a docking molecule for a range of human pathogenic microorganisms. It has been shown that dermatan sulphate mediates the adhesion of *Penicillium marneffei* to the host's ECM (Penc *et al.*, 1999), of *pneumococci* to nasopharyngeal epithelial cells (Srinoulprasert *et al.*, 2006) and of *spirochetes* to fibronectin (Tonnaer *et al.*, 2006). *P. marneffei*, an endemic fungus in Southeast Asia and southern China, is the cause of opportunistic infection in HIV-infected patients who may present with symptoms and signs of the lungs, and abnormal chest radiographs. *P. marneffei* pneumonia does not have specific clinical manifestations, but should be included during differential diagnosis, especially in immunocompromized patients (Deesomchok and Tanprawate, 2006). *Streptococcus* pneumoniae has been recognized as a major cause of pneumonia (Neralla and Meyer, 2004). The pathogenesis of pneumococcal infection is a complex interplay between pneumococcal virulence determinants and the host immune response (Gillespie and Balakrishnan, 2000). Pulmonary hemorrhage represents the main cause of death in severe forms of leptospiral pneumonias due to infection with *spirochetes*. The pathogenesis of the disease involves novel outer membrane proteins and dysregulation of sodium transporters of alveolar epithelial cells, and innate immunity in the development of severe disease (Dolhnikoff *et al.*, 2007).

Keratan sulphate

Keratan sulphate is among the most abundant GAG in airway secretions (Monzon *et al.*, 2006). It is also highly expressed in

the cornea, bones, brain and in the ECM. Keratan sulphate is composed by galactose and N-acetylglucosamine linked together by the action of glycosyl transferases in an alternate pattern (Christner *et al.*, 1979). Sulphation of keratan sulphate, which occurs while a chain is elongated, regulates the organ or cell type-specific function of this GAG (Krusius *et al.*, 1986; Nieduszynski *et al.*, 1990; Degroote *et al.*, 1997; Uchimura *et al.*, 1998). In the ECM, keratan sulphate is linked to the large proteoglycan aggrecan and its length and sulphation increases with age (Praus and Brettschneider, 1975; Brown *et al.*, 1998). Despite the abundance of keratan sulphate in lung secretions, there are no available data regarding its functional role in the lung.

Role of GAG in lung diseases

Altered ECM turnover is a hallmark of several pulmonary diseases, including idiopathic pulmonary arterial hypertension (IPAH), pulmonary fibrosis, asthma or chronic obstructive pulmonary disease (COPD), which underlines the importance of ECM homeostasis for proper lung function (Rabinovitch, 2001; Noble and Jiang, 2006; Laurent *et al.*, 2007). In the lung, the ECM is subjected to a daily turnover of about 10% of total ECM, indicating that subtle changes in turnover rates accumulate to large changes in total ECM composition with time (McAnulty and Laurent, 1987). As GAG are main constituents of the lung ECM, alterations in their synthesis and degradation may reflect to lung pathology.

Idiopathic pulmonary arterial hypertension

IPAH is a rare but fatal disease characterized by elevated blood pressure in the pulmonary circulation, due to increased vascular resistance of pulmonary arterioles (Gaine and Rubin, 1998; Humbert *et al.*, 2004a; Rabinovitch, 2008). If untreated, IPAH leads to right ventricular hypertrophy, failure and subsequent death. Despite major recent advances in the pathophysiology, diagnosis and therapy of IPAH, the molecular mechanisms underlying endothelial and smooth muscle cell dysfunction in this disease remain to be fully elucidated (Humbert *et al.*, 2004b). Early in disease pathogenesis, endothelial cell dysfunction triggers increased vasoconstriction and *in situ* thrombosis (Rabinovitch, 2008). This is followed by pathologic vascular remodelling, a process characterized by intimal fibrosis and thickening of the medial and adventitial layers, due to uncontrolled proliferation of pulmonary arterial smooth muscle cells (PASMC) and perivascular fibroblasts (Olschewski *et al.*, 2001; Humbert *et al.*, 2004b; Eickelberg and Morty, 2007; Rabinovitch, 2008). In parallel, enhanced cellular activation of PASMC and fibroblasts leads to excessive ECM deposition, which potentiates the increased stiffness of pulmonary arteries in IPAH (Rabinovitch, 2001; Hassoun, 2005).

In addition to the pathological changes discussed above, there is increasing interest on the proinflammatory state of the vessel wall in the progression of IPAH (Rabinovitch, 2008). In this respect, additional features of IPAH include the thickening of the pulmonary adventitia and venous hypertrophy (Chazova *et al.*, 1995), increased expression of transforming growth factor (TGF)-β, matrix proteins (such as collagen, elastin, fibronectin, tenascin-C, and GAG) (Jones *et al.*, 1997), macrophages and T cells (Liptay *et al.*, 1993), as well as inflammatory cytokines, such as S100A4 (also known as metastasin 1) (Greenway *et al.*, 2004) and fractalkine (Balabanian *et al.*, 2002). Furthermore, it has been shown that the loss of function of the receptor of bone morphogenic protein (BMP), BMPRII leads to up-regulation of the proinflammatory cytokine IL-6 (Hagen *et al.*, 2007) and that increased expression of the nuclear transcription factor NFATc2, which is associated with inflammatory cells, is observed in T cells from IPAH patients (Bonnet *et al.*, 2007).

It has recently been shown that GAG play a significant role in inflammatory and non-inflammatory lung diseases, exhibiting spatio-temporally distinct effects on epithelial or mesenchymal cell types (Noble and Jiang, 2006; Laurent *et al.*, 2007). As already mentioned, GAG regulate hydration and water homeostasis, maintain cell and tissue structure and function, modulate inflammatory responses and influence tissue repair and remodelling (Noble and Jiang, 2006; Souza-Fernandes *et al.*, 2006). We have recently investigated GAG expression in lungs of patients with IPAH or control transplant donors, and analysed expression and localization of GAG metabolizing enzymes *in vivo* and *in vitro* (Papakonstantinou *et al.*, 2008). We found that IPAH lung tissues exhibit a significantly increased content of HA, the major GAG produced by PASMC. While the relative amount of HA was significantly increased, the content of the sulphated GAG heparan, dermatan or chondroitin sulphate was decreased, indicating an increased ratio of non-sulphated to sulphated GAG. Immunohistochemical staining in tissue sections from lungs of IPAH patients revealed increased HA deposition in areas associated with remodelled pulmonary arteries. The increased HA content of IPAH lung tissues was associated with increased gene expression of HAS 1 and decreased gene expression of HYAL 1. Similar changes were observed in primary human PASMC cultured from the lungs of IPAH patients, which demonstrated a significant decrease in HYAL 1 mRNA levels, compared with PASMC obtained from transplant donor lungs (Papakonstantinou *et al.*, 2008). In addition, stimulation of PASMC with TGF-β1, a growth factor involved in the pathogenesis of IPAH (Humbert *et al.*, 2004b; Eickelberg and Morty, 2007), led to increased HAS 1 gene expression as early as 2 h after stimulation. As increased HA staining was observed in remodeled pulmonary arteries (Papakonstantinou *et al.*, 2008), it is tempting to speculate that HA secretion by PASMC directly influences PASMC proliferation, and may control vasoreactive responses in IPAH. We have previously shown that HA inhibits proliferation of human PASMC (Papakonstantinou *et al.*, 1995; 1998). This effect of HA is mediated via interaction with specific cell surface receptors CD44 or RHAMM (Toole, 2004; Jiang *et al.*, 2007), but also via interaction with toll-like receptors TLR2 and 4 (Jiang *et al.*, 2005). This is substantiated by the fact that selective overexpression of HAS 2 in smooth muscle cells of large and small vessels of transgenic mice resulted in significantly increased HA content in the tunica media of the aorta, enhanced mechanical stiffness and strength, and accelerated development of atherosclerosis (Chai *et al.*, 2005), and that CD44 expression promoted susceptibility to atherosclerosis,

recruitment of macrophages and smooth muscle cell activation in CD44 heterozygote and wild-type mice as compared with CD44-null littermates (Cuff *et al.*, 2001).

It is of interest to note that the biological effects of HA vary depending on its average molecular mass (Turino and Cantor, 2003; Jiang *et al.*, 2007). As mentioned above, under physiological conditions, HA is a polymer of average molecular mass above of 10^6 Da, exerting its 'sweet' beneficiary effects for normal lung function. In contrast, HA fragments of lower molecular mass are associated to 'bitter' effects, leading to malfunction of the lung (Figure 2). Available evidence indicates that HA fragments accumulate following tissue injury. HA fragments ($0.3–0.5 \times 10^6$ Da) have been reported to prolong the survival of eosinophils *in vitro* (Ohkawara *et al.*, 2000), while HA fragments ($<2 \times 10^5$ Da) induced the expression of cytokines, chemokines or inducible NO synthase by macrophages (McKee *et al.*, 1996), affected ECM turnover in murine alveolar macrophages (McKee *et al.*, 1996) and stimulated TGF-β1 synthesis (Ohkawara *et al.*, 2000). Furthermore, it has been shown that fragmented HA with an average molecular mass of 0.25×10^6 Da induced the expression of inflammatory genes (Tammi *et al.*, 2002), while HA of higher molecular weight exhibited the opposite effect and suppressed chemokine expression (Turino and Cantor, 2003). In this respect, it would be of interest to elucidate whether HA expressed in the vascular system of control donor lung tissue specimens is of different average molecular weight than in IPAH lung tissue specimens.

Our *in vivo* observations on IPAH lung tissues were also supported by experiments investigating the effects of BMP and TGF-β superfamily on GAG synthesis and HA secretion in human primary cultures of PASMC. Among the growth factors involved in vascular pathophysiology, BMP and TGF-β isoforms are pleiotropic mediators that, due to abundant expression of their respective receptors BMPRs and TβRs, exhibit multiple effects on all cells within the vascular system (Pepper, 1997; Blobe *et al.*, 2000; Topper, 2000). Recently, the TGF-β and BMP receptor systems have gained significant attention, as mutations in the gene encoding BMPRII (*BMPR2*) have been linked to familial and sporadic primary pulmonary hypertension (Deng *et al.*, 2000; Thomson *et al.*, 2000; Machado *et al.*, 2001; Newman *et al.*, 2001). In addition to *BMPR2*, mutations in the gene encoding ALK1, a TβRI isoform, have been associated with the appearance of pulmonary hypertension in hereditary hemorrhagic telangiectasia types I and II respectively (Osler-Rendu-Weber syndrome) (McAllister *et al.*, 1994; Johnson *et al.*, 1996; McDonald *et al.*, 2000). We found that TGF-β1, but not BMP-2, significantly stimulated total GAG synthesis and HA secretion, an effect that coincided with an increase in HAS 1 gene expression. The TGF-β1-dependent increase in GAG synthesis was mediated via the Smad and p38 MAP kinase pathways (Papakonstantinou *et al.*, 2008). As TGF-β1 is a potent stimulus for PASMC migration (Eickelberg and Morty, 2007), HAS 1-mediated HA synthesis may represent a necessary step for PASMC activation and migration. Dysregulation of HAS/HYAL expression and/or activity may lead to the generation of HA of different molecular masses with distinct biological effects that potentially contribute to the pathogenesis of IPAH. The aforementioned changes in HAS/HYAL expression, along with the change in HA synthesis and content in IPAH, may also result from tissue hypoxia observed in IPAH. We have previously reported that hypoxia potentiates GAG synthesis induced by TGF-β or platelet-derived growth factor-BB by human primary lung fibroblasts from normal lungs (Papakonstantinou *et al.*, 2000; 2002), suggesting that hypoxia is a synergistic regulator of increased GAG deposition observed in IPAH. In conclusion, our studies demonstrate that synergistic regulation of GAG metabolizing enzymes in favour of accumulation may regulate pathologic remodelling in IPAH by favouring the activation state of PASMC.

Pulmonary fibrosis

Idiopathic pulmonary fibrosis (IPF) is a chronic interstitial lung disease that usually results from injury to the lung parenchyma, increased proliferation of mesenchymal cells and excessive accumulation of connective tissue matrix in the interstitium and intraalveolar space of the lung. After injury, resident alveolar cells, including endothelial/epithelial cells and alveolar macrophages, secrete cytokines, chemokines, adhesion molecules and tissue factor. Under normal conditions, this reaction leads to temporary inflammation, tissue formation, remodelling and, finally, normal tissue repair. However, persistent injury and chronic inflammation may accelerate the fibroproliferative response by promoting the secretion of growth factors from resident lung cells, including alveolar macrophages. These growth factors stimulate the proliferation of fibroblasts and smooth muscle cells and the secretion of ECM components, such as collagen, fibronectin and GAG in the lung (Souza-Fernandes *et al.*, 2006). Further to the increased deposition of ECM molecules in the lung, the relative insufficiency of metalloproteinases, the increased secretion of tissue inhibitors of metalloproteinases and the abnormal function of the fibrinolysis system also play critical roles in the fibroproliferative processes in the lung (Suzuki *et al.*, 2004).

Lung fibrosis is mainly studied using the animal model of bleomycin-induced lung injury (Snider *et al.*, 1978). Such studies have demonstrated a critical role for the HA receptor CD44 in the inflammatory processes (Teder *et al.*, 2002). Clearance of HA fragments is crucial in resolving lung inflammation and is dependent on CD44, which is expressed on haematopoietic cells. CD44 deficiency leads to continuous inflammation, increases mortality rate, results in accumulation of HA, prolongs inflammatory gene expression, decreases clearance of apoptotic neutrophils and impairs the ability to generate active TGF-β1 (Teder *et al.*, 2002). CD44 can also mediate wound microvascular endothelial cell migration on fibrogen and invasion into fibrin matrix (Henke *et al.*, 1996).

We have recently investigated the differential expression of GAG in lung tissue specimens from patients with IPF, as compared with controls (lung donors) (Papakonstantinou *et al.*, 2006). We identified the presence of HA, heparan sulphate, dermatan sulphate and chondroitin sulphate both in IPF and control lung tissue specimens. Electrophoresis of total GAG showed that there was a significant increase in the relative amount of HA in IPF, as compared with controls. This increase was about 300%, as also confirmed by ELISA. The

observed increase of HA was also associated with changes in gene expression of HAS 2 and 3 and HYAL 1–3, indicating that there is a differential turnover of HA in IPF. As HA is a key molecule mediating important cellular functions, our results indicate that the increased expression of HA in IPF may be associated with the pathology of the disease.

Asthma – chronic obstractive pulmonary disease

Recent reports on asthma and COPD research provided clear evidence that the pathologies of both diseases cannot be solely explained on the basis of a deregulated immune-response. Malfunction of structure-forming cells and disturbance of the homeostasis of ECM molecules contribute significantly to the pathology of both diseases and reflect to airway remodelling (Johnson *et al.*, 2001; Postma and Timens, 2006). Tissue remodelling describes the structural alterations that occur in the lung due to prolonged chronic inflammation within the airways, and involves qualitatitive or quantitative changes in cell density and the composition of the ECM in the pulmonary epithelium, the basement membrane and the submucosa. Consequently, this modification in the ECM affects airway resistance, compliance and elasticity, leading eventually to loss of lung function (Johnson *et al.*, 2001; Postma and Timens, 2006).

It has been suggested that HA plays a protective role in the bronchial tissue as it could block bronchial obstruction induced by aerosol administration of pancreatic elastase in sheep (Scuri *et al.*, 2001). HA also interacts with tissue kallikreins, which is a multiple family of serine proteinases that are secreted by serous cells of tracheobronchial submucosal glands (Forteza *et al.*, 1999) and generate kallidin (lysyl-bradykinin) from low- and high-molecular-weight kininogen (Lauredo *et al.*, 2004). In the lung, the generation of kallidin and its cleavage product bradykinin can contribute to the pathophysiology of asthma (Baumgarten *et al.*, 1986; Christiansen *et al.*, 1992). Both peptides cause vasodilatation (Tadjkarimi *et al.*, 1992) and increase in vascular permeability (Bell and Wainer, 1983) and bronchoconstriction (Scuri *et al.*, 2000). *In vitro* experiments have demonstrated that HA can inactivate tissue kallikrein, suggesting that the protective effects of HA against elastase-induced bronchoconstriction are mediated through the inactivation of tissue kallikrein (Forteza *et al.*, 1999).

In this context, inhaled HA has been studied in humans as a protective agent against exercise-induced bronchoconstriction (Kunz *et al.*, 2006; Petrigni and Allegra, 2006). However, the results of such studies are contradictory. Petrigni and Allegra (2006) in a randomized, cross-over, single-blind study design have shown that administration of HA ($0.4–4 \times 10^6$ Da) in aerosol form significantly reduces the bronchial hyperreactivity to muscular exercise in asthmatics. The authors concluded that such effect could be attributed to the unique physicochemical properties of HA in the remodelling process. At the same time, it was demonstrated that a single dose of HA administered before exercise did not protect against exercise-induced bronchoconstriction in asthmatic patients. The authors suggested that this may be due to the small size (0.15×10^6 Da) of HA that they have used, which actually increased the degree of airway inflammation in asthmatic patients (Kunz *et al.*, 2006).

The argument for a 'sweet' protective role of HA of high molecular mass in asthma is further supported by reports that HA of high but not of low molecular mass inhibited the function of alveolar (Negrini *et al.*, 2006) and peritoneal macrophages (Farias *et al.*, 2005). In contrast, a 'bitter' role for HA of low molecular mass is indicated by reports that goblet cell metaplasia induced by reactive oxygen species in normal human bronchial epithelial cells was associated with HA depolymerization (Iozzo, 1998). Recent experiments in our laboratory using primary airway smooth muscle cells from healthy lung tissue (control) and from patients with asthma or COPD have shown that there is decreased expression of HA in airway smooth muscle cells from patients with asthma and COPD, as compared with controls. Furthermore, HA in asthma and COPD is of lower molecular mass than HA in controls. Of great interest is our observation that the use of corticosteroids and β_2-agonists enhanced synthesis of HA to control levels (Klagas *et al.*, 2009).

Heparin has been shown to protect against exercise-induced bronchoconstriction (Ahmed *et al.*, 1993; Garrigo *et al.*, 1996). The authors suggested that this effect may be due to prevention of mediator release rather than a direct effect on smooth muscle. Furthermore, heparin may be capable of modulating the extent of remodelling of the airway wall seen in asthma, by modulating the actions of a range of proteins, including ECM proteins, growth factors and certain enzymes, and by inhibiting the proliferation of lung fibroblasts and airway smooth muscle cells (Tyrell *et al.*, 1995). Additionally, heparin is released in the airways physiologically as a homeostatic mechanism to limit the extent of the cellular adhesion and diapedesis. Thus, the release of heparin provides a plausible homeostatic mechanism to limit tissue damage and remodelling following an inflammatory insult to the mucosal surface (Page, 1997).

The 'sweet' and 'bitter' effect of GAG in the pathophysiology of organs other than the lung and relevant pharmacotherapy

The 'sweet' or 'bitter' effect of GAG reported above for lungs appears to transcend to the pathophysiology of other organs. Some of the recent data regarding the effect of GAG in animals or therapeutic uses in humans for diseases other than pulmonary are presented herewith.

Hyaluronic acid

It has been reported that high-molecular-weight HA inhibits neo-intimal macrophage influx after balloon-catheter induced injury in the cholesterol-fed rabbit (Ferns *et al.*, 1995). With respect to humans, it has been reported that HA ophthalmic viscoelastic devices are used in corneal transplantation and vitreoretinal, anterior segment and glaucoma surgery, within the eye and on the surface of the eye to prevent dryness and to facilitate wound healing (Balazs,

2008). Similarly, scleral implants of HA have been reported to reduce the intraocular pressure-lowering effect and complication rate of nonpenetrating deep sclerectomy in primary open angle glaucoma (Russo *et al.*, 2008). In patients with rheumatoid arthritis, intraarticular administration of HA has been reported to alleviate the pain associated with knee (Chou *et al.*, 2008) and ankle joints (Cohen *et al.*, 2008) and to significantly decrease the rate of deterioration of joint structure (Migliore and Granata, 2008). In patients with interstitial cystitis it has been reported that oral or intravesical administration of HA reduced bladder inflammation and 'replenized' the GAG layer (Theoharides *et al.*, 2008), and that intravesical administration of HA is regarded as a safe and efficacious method of treatment of patients with interstitial cystitis/painful bladder syndrome (Porru *et al.*, 2008).

Interactions of HA with CD44 induce activation of receptor tyrosine kinases and promote 'bitter' effects, such as drug resistance in a variety of cancer cell types, including breast, lung and head and neck carcinomas, and lymphoma (Misra *et al.*, 2005; Wang and Bourguignon, 2006; Cordo Russo *et al.*, 2008). An anticipated 'sweet' effect of small HA oligomers, acting as antagonists to high-molecular-weight HA–CD44 interactions, may be proved to be useful in therapeutic strategies aimed at preventing tumour recurrence from therapy-resistant sub-populations within malignant cancers (Toole and Slomiany, 2008).

Heparan sulphate

Intraperitoneal administration of heparanase, an endo-beta-D-glucuronidase that cleaves heparan sulphate saccharide chains, ameliorated the clinical signs in a murine non-obese diabetic model (T cell-dependent disease) of type 1 diabetes by inducing a shift from a Th1- to Th2-phenotype (Bitan *et al.*, 2008). Heparan sulphate also exerts anti-inflammatory effects. Syndecan-1 is a major heparan sulphate proteoglycan. Syndecan-1-null mice showed significantly increased liver injury, vascular permeability and death in response to staphylococcal enterotoxin B challenge compared with wild-type mice. Administration of heparan sulphate rescued syndecan-1-null mice from staphylococcal enterotoxin B induced lethal toxic shock by suppressing the production of TNF-α and IL-6, and attenuating inflammatory tissue injury (Hayashida *et al.*, 2008). In addition, polysulphated molecules, as the family of heparan mimetics, have been reported to have significant therapeutic potential for prion diseases (Ouidja *et al.*, 2007). In humans, it has been reported that heparanase directly contributes to prostate tumour growth in bone and its ability to metastasize to distant organs and that local *in vivo* silencing by small interfering RNA of heparanase resulted in a fourfold inhibition of prostate tumour growth, suggesting that anti-heparanase strategy may become an important modality in the treatment of prostate cancer patients, particularly those with bone metastases (Lerner *et al.*, 2008).

Heparin

Administration of enoxaparin, which is a low-molecular-weight heparin, in patients with diabetic nephropathy exerted antiproteinuric effect not via the renin-angiotensin-aldosterone system, but apparently by inducing intrinsic alterations in the glomerular filter (Benck *et al.*, 2006).

Chondroitin sulphate

Administration of chondroitin sulphate rich in the specific epitope GlcUA-GalNAc (4, 6-O-disulphate) or a specific decasaccharide fraction of this epitope inhibited the metastasis of Lewis lung carcinoma-derived LM66-H11 cells to lungs in the mouse (Li *et al.*, 2008). Also, administration of chondroitin-4-sulphate reduced the effects of caerulein-induced acute pancreatitis in the mouse experimental model, by blocking NF-kappaB and caspase activation due to its antioxidant activity (Campo *et al.*, 2008a). With respect to spinal cord injury, chondroitin sulphate proteoglycan plays a key role during the acute recovery stage in mice, and the sugar component of the proteoglycan is responsible for the inhibitory role of these compounds in axonal regeneration. It has been reported that chondroitin sulphate proteoglycan is considered to be a major obstacle for central nervous system recovery after spinal cord injury in mice due to its well-known activity in limiting axonal growth (Rolls *et al.*, 2008). Administration of chondroitinase ABC increased corticospinal axon growth in organotypic cocultures of brain cortex and spinal cord (Nakamae *et al.*, 2008), while intrathecal administration of chondroitinase ABC in a rat model with spinal cord injury caused a slight decrease in chondroitin sulphate proteoglycans in the scar and a minimal increase in sensory axonal regeneration into and across the laceration gap (Shields *et al.*, 2008). On the same line, inhibition of chondroitin sulphate proteoglycan synthesis by xyloside immediately after injury impaired functional motor recovery and increased tissue loss after spinal cord injury in mice (Rolls *et al.*, 2008).

The 'sweet' role of chondroitin sulphate proteoglycan during the acute stage and its 'bitter' effect at later stages emphasizes the need to retain the endogenous potential of this molecule in repair by controlling its levels at different stages of post-injury repair (Rolls *et al.*, 2008). With respect to arthritis, it has been reported that administration of chondroitin sulphate in a rabbit model of atherosclerosis aggravated by chronic arthritis reduced the concentration of the proinflammatory molecules C-reactive protein, IL-6 and the nuclear translocation of NF-kappaB and inhibited the expression of CCL2/monocyte chemoattractant protein-1 and cyclooxygenase-2. Moreover, chondroitin sulphate decreased the percentage of rabbits with atherosclerosis and chronic arthritis that developed vascular lesions in the aorta (Herrero-Beaumont *et al.*, 2008). Similarly, intraperitoneal administration of chondroitin-4-sulphate in mice with collagen-induced arthritis ameliorated all the symptoms of inflammation in the articular knee and paw joints, limited lipid peroxidation, inhibited NF-kappaB activation, decreased mRNA MMP-13 and caspases expression of and their related protein, restored endogenous antioxidants and reduced PMN accumulation in the damaged cartilage (Campo *et al.*, 2008b).

In patients with osteoarthritis, administration of 800 mg of chondroitin sulphate for 3 month mode, twice a year, has been recommended by the European League Against Rheumatism for the treatment of knee osteoarthritis as evidence 1A and strength of recommendation A, which represents the

highest level for a therapeutic strategy (Uebelhart, 2008). However, data provided by meta-analyses published between 1997 and 2007 indicate that chondroitin sulphate has a slight to moderate efficacy in the symptomatic treatment of osteoarthritis, with an excellent safety profile (Monfort *et al.*, 2008).

Further application of chondroitin sulphate in humans refers to eye surgery, where chondroitin sulphate is topically applied for phacoemulsification postoperatively (Praveen *et al.*, 2008). In humans, it has also been reported that instillation of chondroitin sulphate effectively reduced all symptoms in patients with chronic forms of cystitis (Nordling and van Ophoven, 2008), that oral or intravesical administration of chondroitin sulphate reduced bladder inflammation and 'replenished' the GAG layer (Theoharides *et al.*, 2008) and that intravesical administration of chondroitin sulphate is regarded as a safe and efficacious method of treatment of patients with interstitial cystitis/painful bladder syndrome (Porru *et al.*, 2008). Further effects of chondroitin sulphate are associated in humans with coagulation. Odiparcil, an orally active beta-d-thioxyloside analoglue, exerts antithrombotic activity apparently by increasing circulating endogenous levels of chondroitin sulphate-related GAG (Myers *et al.*, 2008). However, contaminated heparin with oversulphated chondroitin sulphate has been associated with severe anaphylactoid reactions via activation of the contact system after intravenous heparin administration (Kishimoto *et al.*, 2008).

Dermatan sulphate

It has been reported that administration of dermatan sulphate is an effective anticoagulant in patients on continuous renal replacement therapy (Vitale *et al.*, 2008).

Keratan sulphate

Increase in serum keratan sulphate was associated with progression of osteoarthritis (Tang *et al.*, 2008).

Cancer

With respect to cancer, a growing body of evidence supports crucial roles of GAG at various pathophysiological steps of tumour progression. GAG regulate tumour proliferation, invasion, haematogenous metastasis and angiogenesis, and increased understanding of these roles sets the stage for developing pharmaceutical agents that target these molecules. Such novel agents might be used alone or in combination with operative and/or chemoradiation strategies for treating cancer (Fuster and Esko, 2005). For example, heparan sulphate proteoglycans allow primary tumour growth and angiogenesis through growth factor-dependent signalling (Fuster and Esko, 2005). It might be possible to target tumour cells by developing agents that bind heparan sulphate or influence its synthesis (Bishop *et al.*, 2007).

Vaccines

There are various vaccines used made of viral coat carbohydrates against microorganisms responsible for infectious diseases and other conditions, such as *Salmonella typhi* – Typhus, *Sreptococcus pneumoniae* – Pneumonia, *Neisseria meningitides* – Bacterial meningitis, *Haemophilus influenza* type b – Influenza or various vaccines under development that use surface glycolipid against *Plasmodium falciparum* – Malaria and *Leishmania* – Leishmaniasis, or finally the monosaccharide oseltamivir phosphate against *Influenza* (Verez-Bencomo *et al.*, 2004; Seeberger and Werz, 2007; Oppenheimer *et al.*, 2008).

Conclusion

There is increasing evidence over the last decade that GAG are involved in the pathophysiology of the lung and other organs. Of particular interest is the fact that the same molecule may exert a beneficiary or a detrimental effect, depending on the size, as is the case of HA, or the degree of sulphation, as is the case of heparin. This reciprocal transition from the 'sweet' to the 'bitter' involvement of GAG in lung function is apparently mediated by specific enzymes responsible for the synthesis or degradation of GAG, rendering both the end GAG product as well as the liable enzymes as putative targets for treatment of specific lung diseases. Advances in carbohydrate and GAG synthesis, analysis and manipulation through the fields of glycochemistry and glycobiology and new technologies using carbohydrate and lectin microarrays offer improved diagnostic and drug development possibilities that are fascinating for the pharmacologist.

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