

The role of proteoglycans in the regulation of airways inflammation and airways remodelling

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Proteoglycans such as heparin have been recognized as useful anticoagulant drugs for a long time. However, it is now apparent that proteoglycans possess a wide array of biologic activities that are of potential interest in our understanding of the regulation of airways inflammation and remodelling in addition to their anticoagulant effects.¹ A proteoglycan is any glycoprotein that consists of a protein core and has glycosaminoglycan side chains. Glycosaminoglycans are polysaccharides that have a simple repeating disaccharide unit and are highly anionic in nature because of the presence of many sulphate and carboxyl groups.² Heparin is found exclusively in mast cell granules in mammals,³ whereas heparan sulphate is ubiquitous, being found on the surface of most cell types, particularly on the surface of vascular endothelial cells.¹ However, although we have known for some time that heparin is present in mast cell granules, where it binds histamine and the mast cell enzymes, tryptase, and chymase,³ little is known about the role of endogenous heparin. It is now clear that after the exposure of human lung mast cells to house dust-mite allergen, heparin can be released into the extracellular environment.⁴ This article will review the biologic activities of heparin and related proteoglycans that are of interest in the context of our understanding of airway inflammation and remodelling.

AIRWAYS INFLAMMATION

Heparin is now known to bind many of the chemokines that have proinflammatory actions on inflammatory cells and to interfere with the activities of many of the adhesion molecules expressed on endothelial cells and/or leukocytes.¹ In particular, heparin will bind IL-8, platelet factor 4, and RANTES,^{5,6} inhibit the chemotaxis of eosinophils induced by these chemokines,⁷ and inhibit

the effects of the adhesion molecules very-late antigen 4,⁸ fibronectin,⁹ and L- and P-selectins.¹⁰ This is of interest because heparin and other proteoglycans have been shown to inhibit adhesion of PMNs to endothelial cells in vitro in response to a variety of inflammatory stimuli.¹ Furthermore, heparin will inhibit recruitment of PMNs into the peritoneal cavity of experimental animals induced by injection of thioglycollate.¹⁰ Recently, we have observed that heparin and a number of other related molecules will inhibit the recruitment of inflammatory cells into the airways of guinea pigs and rabbits in response to either platelet-activating factor^{12,13} or allergen.^{14,15} Neither cell adhesion in vitro¹¹ nor the recruitment of inflammatory cells into the airways in vivo are inhibited by the linear anionic molecule polyglutamic acid, which suggests that the anionic nature of these molecules is not per se responsible for this anti-inflammatory activity of proteoglycans.¹²⁻¹⁵ Heparin has also been shown to inhibit the cytotoxicity of epithelial cells induced by cationic mediators, such as major basic protein and eosinophil cationic protein.¹⁶ It is plausible therefore that the release of heparin in response to antigen antibody interactions may serve as a protective homeostatic mechanism to limit cell recruitment and tissue damage. This suggestion¹⁷ is supported by the observation that heparin can inhibit the biologic activities of other potent inflammatory mediators released by inflammatory cells including oxygen free radicals,¹⁸ elastase,¹⁹ IL-4,²⁰ cathepsin G,²¹ tryptase,²² and chymase.¹³ The ability of heparin to inhibit elastase is also shared with molecules that lack anticoagulant activity such as n-desulphated heparin, which suggests that this biologic activity is unrelated to the ability of heparin to inhibit coagulation factors.²³

Another important inflammatory cell type in allergic inflammatory processes is the T-lymphocyte, and it is of interest therefore that heparin has been shown to influence a number of well-characterized T-lymphocyte-mediated responses.¹ Thus heparin has been shown to inhibit allergic encephalomyelitis,²⁴ delayed hypersensitivity reactions,²⁵ and xenograft transplantation rejection.²⁶ One biologic activity that is of relevance here is the ability of heparin and related molecules to inhibit the heparinase enzyme secreted by lymphocytes.²⁷ This enzyme is able to degrade heparan sulphate on the vessel wall and is thought to provide one of the mechanisms whereby lymphocytes can cross endothelial cells and traffic into tissues. Inhibition of heparinase by endogenously released or exogenously administered heparin

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could therefore serve as a mechanism to limit T-lymphocyte-mediated responses. This effect of heparin is also seen with proteoglycans that lack anticoagulant activities, suggesting that this biologic activity is independent of the ability of heparin to inhibit clotting factors.²⁵

BRONCHIAL HYPERRESPONSIVENESS

It is now apparent that one of the consequences of airways inflammation may be exacerbations of bronchial hyperresponsiveness. Heparin has been shown to inhibit bronchial hyperresponsiveness induced by a variety of stimuli including platelet-activating factor,¹³ allergen,^{28,29} and cationic proteins such as major basic protein and platelet factor 4.³⁰ Again, the linear polyanionic molecule polyglutamic acid, unlike heparin, does not inhibit bronchial hyperresponsiveness; thus the ability of heparin to interfere with this process is not solely due to the anionic nature of the molecule.^{13,28} In allergic guinea pigs, heparin has also been shown to reverse allergen-induced bronchial hyperresponsiveness by a mechanism suggested to be related to the inhibition of the effect of eosinophil-derived cationic proteins on muscarinic M₂ receptors on airway nerves.³¹ In allergic sheep, heparin has also been shown to inhibit allergen-induced bronchial hyperresponsiveness by a mechanism that is thought to be the result of the inhibition of mast cell degranulation.²⁹ However, in other species including humans, heparin does not appear to inhibit mast cell degranulation.^{32,33}

AIRWAY REMODELLING

One of the consequences of regular injury to the mucosal surface of the airway is thought to be remodelling of the underlying airway wall structures, such that in individuals with asthma there is evidence of a thickening of the airway smooth muscle,³⁴ along with a thickening of the basement membrane associated with myofibroblast proliferation and subepithelial collagen deposition.³⁵ This is of interest in the context of the pathogenesis of airways diseases because such structural changes may be resistant to current therapy^{36,37} and, on mathematical grounds, have been suggested to contribute to the persistent airways responsiveness that is so characteristic of asthma.³⁸ From studies with vascular³⁹ and intestinal smooth muscle,⁴⁰ it is clear that proteoglycans are very effective inhibitors of smooth muscle proliferation *in vitro* and that heparin at least can inhibit vascular remodelling *in vivo*.⁴¹ Recent studies have suggested that heparin and related proteoglycans can also inhibit the proliferation of airway smooth muscle *in vitro* induced by a variety of stimuli and in a number of species, including human airway smooth muscle.^{42,43} Desulphated molecules and heparin disaccharide, however, do not inhibit airway smooth muscle proliferation, providing evidence for some structural determinants necessary for this antiproliferative activity.⁴² The antiproliferative activity of heparin is also shared with molecules lacking anticoagulant activity.²³

In addition, heparin can inhibit fibroblast proliferation⁴⁴ and will inhibit the secretion of collagen by fibroblasts,⁴⁵ suggesting that heparin could serve to limit the extent of tissue remodelling after injury. It is also clear that many of the basic growth factors, such as fibroblast growth factor⁴⁶ implicated in tissue remodelling, are affected by heparin.

CLINICAL STUDIES

It has been known since the 1920s that paradoxically heparin as a mast cell product could inhibit the mast cell-mediated condition, anaphylaxis.⁴⁷ This raises the possibility that heparin-like molecules could provide a novel way to treat allergic disorders. Certainly, there are studies in the literature that show that heparin can inhibit inflammatory responses,⁴⁸ including allergen-induced airway changes.⁴⁹ More recently, such observations have been extended to show that heparin can inhibit allergen-induced bronchoconstriction in some,⁵⁰ but not all, studies.⁵¹ Heparin has also been reported to inhibit exercise-induced bronchoconstriction, but not oxygen saturation or methacholine-induced bronchoconstriction in individuals with asthma,⁵² which suggests that any protective effects of heparin in the airways are not due to nonselective effects on airway smooth muscle or airway nerves. Heparin has also now been reported to inhibit the allergen-induced late onset responses in individuals with allergic asthma,³² and topical administration of heparin to the nasal mucosa of patients with allergic rhinitis has also been reported to inhibit eosinophil infiltration into the nose induced by allergen exposure.⁵³ This supports the observations made in experimental animals and raises the possibility of topical therapy with proteoglycans for the treatment of allergic rhinitis. Heparin has also recently been reported to inhibit ulcerative colitis⁵⁴ and interstitial cystitis⁵⁵ that are not explained by the anticoagulant effects of this molecule, which suggests that heparin and related proteoglycans may have a broader use in the treatment of inflammatory conditions.

CONCLUSION

There is a growing body of evidence that proteoglycans possess a wide range of biologic activities of relevance to our understanding of inflammatory process. The recent availability of heparin-like molecules that lack anticoagulant activity, but that retain anti-inflammatory and antiproliferative activity,²³ is encouraging because it raises the potential for such molecules to be exploited therapeutically as novel anti-inflammatory drugs that lack one of the properties of heparin that currently limits its wider therapeutic application.

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