PROTEOGLYCANS IN LUNG DISEASE

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18

Heparin as a Potential Therapeutic Agent to Reverse Vascular Remodeling

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I. Introduction

Vascular changes after chronic hypoxia are characterized by hyperplasia, hypertrophy, and migration of smooth muscle cells (SMCs) in the media of muscular and partially muscular pulmonary arteries (1,2). A number of factors are known to cause SMC migration, such as serum, platelet-derived growth factor (PDGF)-BB (3,4), transforming growth factor-β (5), fibrinogen (6), oxidized low-density lipoprotein (7,8), and angiotensin II (9,10).

Heparin, discovered nearly 80 years ago (11), is widely used as an anticoagulant drug. Besides its anticoagulation activity, heparin has a variety of other biological and biochemical activities that include the following: [1] regulation of lipid metabolism (12); [2] control of blood fluidity at the endothelial surface (13); [3] control of cell attachment to various proteins in extracellular matrix (ECM) (14–16); [4] binding with acidic and basic fibroblast growth factors (17,18); [5] binding to interleukin-3 and granulocyte-macrophage colony stimulating factor (19,20); and [6] inhibition of serotonin induced pulmonary artery smooth muscle cell hypertrophy (21). Heparin stimulates endothelial cell growth (22), whereas it inhibits the proliferation of renal mesangial cells (23), rat cervical

epithelial cells (24), transformed cell lines (25–27), and systemic and pulmonary artery smooth muscle cells (28,29). Of the types of biological activities just mentioned, anticoagulation has been extensively discussed in several reviews (30–37). Other biological activities have recently been reviewed (38–46).

Regulation of vascular cellular smooth muscle proliferation by heparin was reviewed in 1989 (47), 1994 (48), and 2000 (49). These reviews specifically discuss the domains in heparin responsible for its antiproliferative activity on aortic vascular smooth muscle cells. Briefly, they suggested:

1. The anticoagulant and antiproliferative properties of heparin reside in different heparin domains.
2. The 3-*O*-sulfo group on the internal glucosamine residue of a chemically synthesized pentasaccharide (Fig. 1) (50) is critical for growth inhibitory capacity of the pentasaccharide (51).
3. A dodecasaccharide sequence in heparin contains the full antiproliferative activity (52).
4. A 2-*O*-sulfo group in the glucuronic acid residues of heparin is not essential for antiproliferative activity (53).
5. *N*-acetylation of *N*-desulfonated glucosamine residues does not seem to restore the antiproliferative activity (54).
6. Both *O*-sulfo and *N*-sulfo groups are important for antiproliferative activity, but the relationship between the extent of *N*-desulfonation and the inhibition of cell proliferation is not straightforward (54).

Because vascular remodeling with smooth muscle cell hypertrophy and hyperplasia contribute to the high pulmonary vascular resistance seen in primary as well as secondary pulmonary hypertension, interest continues in heparin as a possible therapeutic agent to reverse vascular remodeling. Efforts have been made to establish the domain within the heparin polysaccharide, which is related to the inhibition of growth of pulmonary artery smooth muscle cell (PASMC). In studies aimed at understanding heparin's structure-activity relationship (SAR), a

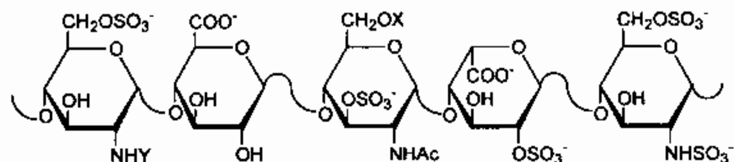


Figure 1 Antithrombin-binding, anticoagulant pentasaccharide demonstrating a structure critical for growth inhibition. Where X = H or SO_3^- and Y = SO_3^- or Ac. The central residue has a unique 3–*O*–sulfo group.

Table 1 General Composition of Different Glycosaminoglycans

Glycosaminoglycan	Saccharide backbone	*N*-acetyl	SO_3^-
Hyaluronan	→4)-β-D-GlcA(1→3)-β-D-GlcN(1→	1	0
Chondroitin sulfate	→4)-β-D-GlcA(1→3)-β-D-GalN(1→	1	1
Dermatan sulfate	→4)-α-L-IdoA(1→3)-β-D-GalN(1→ [→4)-β-D-GlcA(1→]	1	1
Heparan sulfate	→4)-β-D-GlcA)(1→4)-α-D-GlcN(1→ [→4)-α-L-IdoA(1→]	<1	0–2
Heparin	→4)-α-L-IdoA(1→4)-α-D-GlcN(1→ [→4)-β-D-GlcA(1→]	≪1	2–3

GlcA = glucuronic acid; IdoA = iduronic acid; GlcN = glucosamine; GalN = galactosamine; residue shown in brackets is minor component.

variety of methods including chemical modification, fractionation, and enzymatic and chemical degradation have been employed (55,56).

II. Heparin Structure

A. Arrangement of Sugars in Glycosaminoglycan Chains

Heparin is a member of a class of acidic polysaccharides called glycosaminoglycans (GAGs) (Table 1) (57). Heparin consists of alternating residues of uronic acid (either α-L-iduronic acid [major] or β-D-glucuronic acid [minor]) and hexosamine (α-D-glucosamine) connected through 1→4-glycosidic linkages and covalently bound to serine residues of the serglycin core protein. Heparin has *O*-sulfo, *N*-sulfo, and *N*-acetyl substituents that are usually distributed in a heterogeneous array along the GAG chains. Heparan sulfate is a structurally related GAG, having a reduced content of *O*- and *N*-sulfo groups and iduronic acid (Table 1).

B. Protein Core

The protein cores of heparan sulfate are diverse and heterogeneous, varying in size from 20 to 150 kDa and appear to share only the capacity to bear GAG chains. Repetitive serine-glycine sequences are found in the protein core of the proteoglycan carrying the heparin chain. The GAG chains are connected to the protein core through a tetrasaccharide linkage region (Fig. 2) (57). Heparin helicity results in a secondary structural pattern or coiling of the GAG chains (58) (Fig. 3). Heparin's secondary structure, in conjunction with its chirality (i.e., configuration of the carbon atoms at the asymmetric centers), inherent in the constituent monosaccharides, makes heparin a uniquely complex macromolecule.

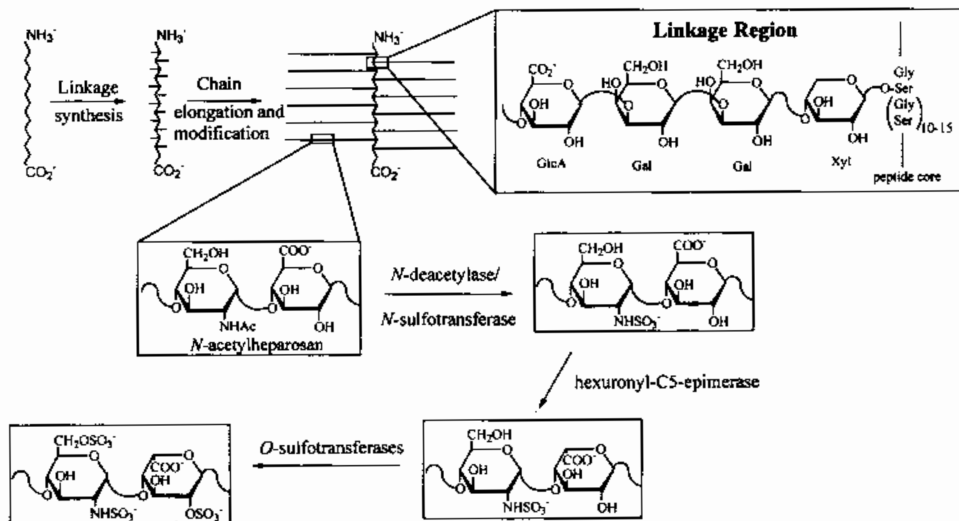


Figure 2 The biosynthesis and structure of the heparin proteoglycan. The linkage region tetrasaccharide as well as key intermediate disaccharide structures formed during heparin biosynthesis are shown in boxes.

III. Low-Molecular-Weight Heparins

Low-molecular-weight (LMW) heparins, currently used in treatment of acute proximal deep-vein thrombosis, are obtained by the controlled enzymatic or chemical depolymerization of heparin (56). In the past decade several LMW heparins (Table 2) have been prepared and approved by the U.S. Food and Drug Administration. These LMW heparins, approximately one-third the size of heparin, show improved pharmacokinetic and pharmacodynamic profiles.

Figure 3 The structure of a heparin dodecasaccharide sequence obtained from NMR (58), studies show the helicity of the heparin polymer.

Table 2 Low-Molecular-Weight Heparins

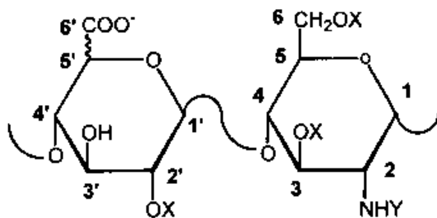
Trade name(s)	Approved name(s)	Manufacturer(s)	Method(s) of production
Fluxum	Parnaparin sodium	Opocrin S.p.a., Alfa Wasserman	Peroxidolysis
Fragmin	Dalteparin sodium	Pharmacia Upjohn	Deaminative cleavage
Fraxiparin	Nadroparin sodium	Sanofi Recherche	Deaminative cleavage
Logiparin	Tinzaprin sodium	Novo Nordisk	Enzymatic β-elimination
Lovenox	Enoxaparin sodium	Aventis	Chemical β-elimination
Normiflo	Ardeparin sodium	Wyeth-Ayerst Research, Pharmacia, Hepar	Peroxidolysis
Sandoparin	Certiparin sodium	Sandoz AG	Deaminative cleavage

LMW heparins directly inhibit the intrinsic factor Xa activity complex (intrinsic tenase) but have no effect on the pro-thrombic activity (pro-thrombinase). In order to understand the role of sulfo group on these activities, LMW heparin was *N*-desulfonated and hypersulfonated separately. *N*-desulfonation of LMW heparin reduced its affinity for antithrombin. In contrast, hypersulfonation enhanced both the intrinsic tenase and pro-thrombic inhibitory activities. Hence, the biological activity of these heparin derivatives showed that they act as potent antithrombin-independent inhibitors of coagulation by attenuating intrinsic tenase and depending on the sulfonation may also inhibit prothrombinase (59).

IV. Differences in the Structure of Heparin and Heparan Sulfate

Heparin is uniquely found in the intracellular granules of certain mast cells, while heparan sulfate is ubiquitously distributed in the extracellular environment. Heparin and heparan sulfate originate from the same biosynthetic precursor, *N*-acetylheparosan (Fig. 2). Following the initial assembly of the *N*-acetylheparosan polymer from monosaccharide precursors, biosynthesis proceeds much further for heparin than for heparan sulfate (60–64). These additional biosynthetic steps for heparin result in a glycosaminoglycan with a higher content of *N*-sulfo groups, iduronate, and *O*-sulfo groups than heparan sulfate (65).

Both heparin and heparan sulfate demonstrate substantial sequence heterogeneity (Fig. 4). Two carbon atoms on each glucosamine residue (positions 3 and 6) and one carbon on each uronic acid residue (position 2′) can contain an *O*-sulfo group. One carbon (position 2) on the glucosamine residue can contain either an *N*-acetyl or *N*-sulfo group, and the uronic acid residue can be isomeric (position 5) giving rise to either glucuronic or iduronic acid. Forty-eight different



Four uronate residues

2'X	5'
hydrogen	iduronate
sulfo	iduronate
hydrogen	glucuronate
sulfo	glucuronate

Twelve glucosamine residues

2Y	3X	6X
sulfo	hydrogen	hydrogen
sulfo	hydrogen	sulfo
sulfo	sulfo	hydrogen
sulfo	sulfo	sulfo
acetyl	hydrogen	hydrogen
acetyl	hydrogen	sulfo
acetyl	sulfo	hydrogen
acetyl	sulfo	sulfo
hydrogen	hydrogen	hydrogen
hydrogen	hydrogen	sulfo
hydrogen	sulfo	hydrogen
hydrogen	sulfo	sulfo

Figure 4 The possible 48 disaccharide sequences that can be found in heparin and heparan sulfate are shown. Many, but not all, of these sequences have been reported to date (From Refs. 66 and 67).

disaccharide structures can result from the combination of the different monosaccharide residues in heparin and heparan sulfate GAG chains (Fig. 4).

V. Heparin and Pulmonary Hypertension

Heparin is released by endothelial cells, and hypoxia increases this release (68). Heparin thus has the potential to regulate pulmonary vascular growth and remodeling.

The effect of heparin on pulmonary hypertension and the associated vascular remodeling has been reviewed by us (69). We have shown in a mouse model of chronic hypoxia, that heparin inhibited the medial smooth muscle increase in vessels associated with terminal bronchioles, reduced right ventricular systolic pressure, and partially prevented the increase in medial thickness of intracinar vessels after 26 days of hypoxia (70). Heparin had no effect on the hematocrit and was effective at low doses that did not prolong the partial thromboplastin

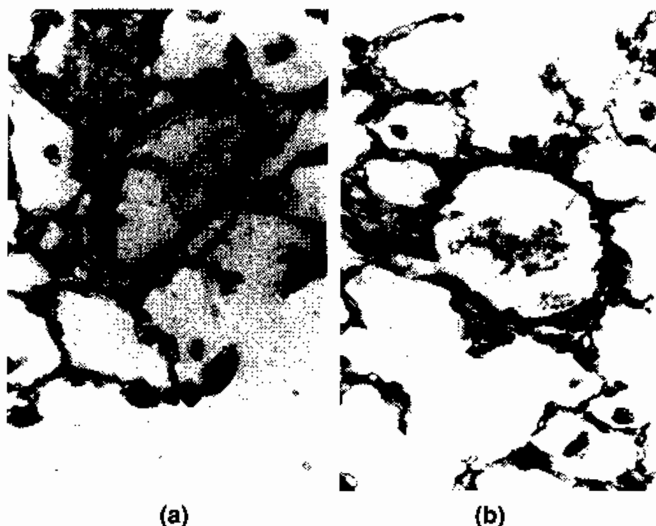


Figure 5 Light micrograph showing: (A) Distal pulmonary artery with two discernible elastic laminae and a thick media from a hypoxic control animal. The artery is adjacent to a terminal bronchiole. (B) Distal pulmonary artery also with two elastic laminae from a heparin-treated hypoxic animal. Here the media is significantly thinner. (Elastin stain; original magnification: × 125.) (From Ref. 72.)

time. Heparin did not block the rise in right ventricular systolic pressure after acute hypoxia, indicating that heparin prevented vascular remodeling through a mechanism that did not involve blockade of hypoxic vasoconstriction.

Subsequently we showed in a guinea pig model of chronic hypoxia pulmonary hypertension in which we could measure cardiac output (71), that certain commercial heparin preparations given by continuous subcutaneous infusion resulted in 50% reduction in medial thickness of alveolar duct vessels (Fig. 5) and completely prevented the medial smooth muscle increase in vessels associated with terminal bronchioles (Fig. 6) (72). Cardiac output was unaffected by the heparin. Moreover, we found that fully established hypoxic pulmonary hypertension in the guinea pig was substantially reversed by heparin (73) and that heparin administration by aerosol was effective (74). We have also shown that different heparin lots even from the same company vary in their ability to inhibit SMC proliferation and hypertrophy (21) and that this variation correlates with the ability of these heparins to prevent hypoxic pulmonary hypertension (29). Rats were said to be resistant to heparin, but we found that a strongly antiproliferative heparin was effective in rats (75).

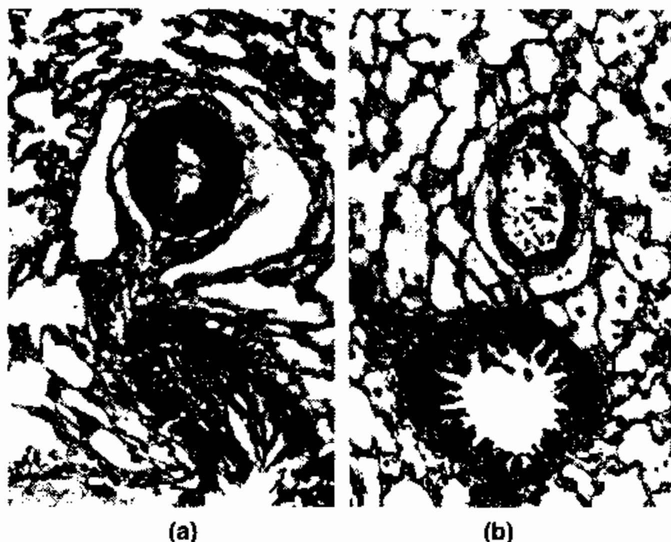


Figure 6 Light micrograph showing: (A) Distal pulmonary artery accompanying an aveolar duct from a hypoxic control animal. Two elastic laminae are seen, separated by a thickened media. (B) Distal pulmonary artery accompanying an alveolar duct from a heparin-treated hypoxic animal. There is scant smooth muscle present in between the two elastic laminae. (Elastic stain; original magnification: × 500.) (From Ref. 72.)

VI. Antiproliferative Activity of Heparin and Its Derivatives

A. Mechanisms Contributing to Heparin Inhibition of Smooth Muscle Cell Growth

Heparin is a potent inhibitor of smooth muscle cell (SMC) proliferation (29,76–78), and this is true of both anticoagulant and nonanticoagulant forms (79). Although much attention has been focused on factors that stimulate SMC proliferation (80), very little is known about the mechanisms maintaining these cells in a quiescent state, or about the reestablishment of a quiescent state after their proliferative response has been initiated.

Circulating heparin binds to endothelial cells and is taken up by the reticuloendothelial system where it enters a cellular pool to be released at a later stage (81). Heparin also binds to specific binding sites on smooth muscle cells and is internalized (82). Some antiproliferative effects are mediated by this specific binding, although it is not clear whether internalization is essential. Heparin blocks the cell cycle at either the G_0/G_1 transition point (83) or at mid to late G_1 progression (82–85), and may inhibit such cellular intermediate processes as pro-

Table 3 Properties of Heparins from Different Manufacturers

	USP[a] (U/mg)	Antiproliferative activity[b]	Protein content[c]	Amino acid[d]	Hexosamine[e]	
					GlcN	GalN
Upjohn	140	48	1.56	DTSEGAYFKH	26.3	none
Elkins-Sinn	180	12	0.17	SGMYFKH	24.4	0.23
Choay		0	1.57	DT(tr)S(tr)EGMFKHR	4.38	none

[a] USP units of anticoagulant activity from the manufacturer.
[b] Percent inhibition of bovine pulmonary artery smooth muscle cell growth in vitro at a concentration of 1.0 μg/mL.
[c] Percent of the total heparin (w/w).
[d] Amino acids present in the core protein of heparin.
[e] Percent w/w.

tein kinase C activation, c-fos and c-myc induction (86,87), activator-protein-1 (AP-1)/fos-jun binding activity and posttransitional modification of jun B (88–90). Heparin has also been shown to selectively block the protein kinase C pathway of mitogenic signaling (91) and the phosphorylation of mitogen-activated protein kinase (MAPK) (92).

We have demonstrated that pulmonary artery smooth muscle cell (PASMC) mitogens such as platelet-derived growth factor (PDGF) and epidermal growth factor (EGF) act through the Na^+/H^+ antiporter, by stimulating a one-for-one exchange of extracellular Na^+ for intracellular H^+, to cause intracellular alkalinization, a permissive first step for cell division (93). Further, we have demonstrated that antiproliferative heparins block Na^+/H^- exchange in a manner directly related to antiproliferative activity (94).

B. Structure-Activity Relationship (SAR)

To understand the SAR of the heparin polysaccharide, we compared the antiproliferative activity of three commercially available heparins. These preparations were from Upjohn, Elkins-Sinn, and Choay Pharmaceuticals. The growth inhibition activity of these heparins on pulmonary artery smooth muscle cells varied and was in the order: Upjohn > Elkins-Sinn > Choay, respectively (94). The properties of these heparin preparations are summarized in Table 3.

C. Influence of Molecular Weight, Protein Core, and Heparin GAG Chains on Antiproliferative Activity

SAR studies carried out by preparing discrete sizes of antiproliferative heparin fragments by chemical modification of heparin show that dodecasaccharide and

larger fragments had maximal antiproliferative activity (52,95). Further, Tiozzo and coworkers (96) demonstrated that the reduction of the molecular weight of heparin is associated with a progressive reduction of the antiproliferative activity. These studies were based on chemically modified heparin.

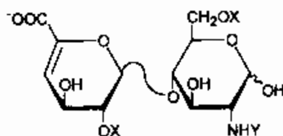
In recent years, we assessed the influence of molecular weight (MW), protein core, and GAG chain of native heparin on PASMC proliferation (96). We fractionated highly potent Upjohn heparin by dissolving it in water and dialyzing it against water without chemical depolymerization. We then lyophilized both dialyzate, having a LMW (<3.5 kDa) and retentate having a high molecular weight (HMW) (>3.5 kDa). The residual core peptide of the Upjohn heparin was isolated by exhaustive treatment with heparin lyase I and III (97). GAG chains of the Upjohn heparin were liberated with alkaline borohydride treatment (98). No appreciable difference on the growth inhibition of PASMC between LMW and HMW heparin fractions was found. The protein core showed no antiproliferative activity. The GAG chains showed a similar inhibition on the growth of PASMC as that of parent heparin. These data suggest that the antiproliferative properties of heparin reside in the GAG chain and not in core protein. These data also suggest that both HMW and LMW heparin fractions have sufficient *N*- and *O*-sulfo groups in their carbohydrate residues necessary for the antiproliferative effect.

D. 3-*O*-Sulfonation of Glucosamine Residue Is Not Critical for Antiproliferative Activity in Full-Length Heparin

Three commercially available heparins were degraded with heparin lyase I and III to evaluate their overall content of 3-*O*-sulfo group containing glucosamine residues to see if 3-*O*-sulfo group content correlated the antiproliferative effect of the three heparins. These enzymes are unable to degrade intermediate heparin oligosaccharides containing 3-*O*-sulfo groups in a glucosamine residue into disaccharide units. Instead, tetrasaccharides containing 3-*O*-sulfo groups were formed (99,100). Thus, the content of heparin lyase resistant tetrasaccharides in the digest correlated with the content of 3-*O*-sulfo groups.

The oligosaccharide profiles of the three different pharmaceutical heparins after digestion with heparin lyase resistant I and III demonstrated that the most potent heparin (Upjohn) contained the least amount of heparin lyase resistant tetrasaccharide and hence the least amount of 3-*O*-sulfo groups on glucosamine residues. These results suggest that the presence of a 3-*O*-sulfo group in pharmaceutical heparin was not an essential requirement for antiproliferative activity, as previously reported (51) based on data derived from the synthetic pentasaccharide (50). The unsaturated disaccharides liberated after heparin lyase treatment of these three heparins were analyzed by us, and results are presented in Table 4 (97). We showed that the most potent Upjohn heparin preparation had the

Table 4 Disaccharide Composition of Heparins from Different Manufacturers



Disaccharide	X^6	Y^2	$X^{2'}$	Upjohn	Elkins-Sinn	Choay
1	H	Ac	H	0.9	3.9	14.5
2	H	SO_3^-	H	0.3	2.0	—
3	SO_3	Ac	H	—	3.9	—
4	H	Ac	SO_3	—	1.7	—
5	SO_3	SO_3	H	5.4	11.5	—
6	H	SO_3	SO_3	4.8	6.3	18.0
7	SO_3	Ac	SO_3	0.4	1.5	1.5
8	SO_3	SO_3	SO_3	86.8	66.3	66.0

Disaccharides were released by treating exhaustively with heparin lyase I and III. Results are expressed as percent of total disaccharides released.

largest amount of a highly sulfated unsaturated disaccharide (Table 4, disaccharide **8**).

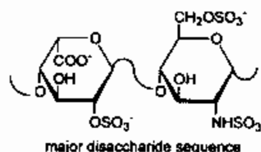
E. Effect of Sulfonation of Polysaccharides on Antiproliferative Activity

The effect of *N*- and *O*-linked sulfo groups on glucosamine and uronic acid sugar residues in heparin on antiproliferative activity has been studied in several laboratories. Tiozzo and coworkers (54) modified heparin to produce *N*-desulfo and *O*-desulfo heparin derivatives and found that the 2-*O*-sulfo group in heparin was important for antiproliferative properties (54). Wright and coworkers (53), on the other hand, reported that the 2-*O*-sulfo group in heparin was not essential for antiproliferative activity. To clarify the role of *N*- and *O*-sulfo groups for PASMC growth inhibition, we prepared fully *O*-sulfonated heparin, heparan, chondroitin sulfate, dermatan sulfate, and hyaluronan using sulfur trioxide (Fig. 7) (101,102). *N*-sulfonated acharan sulfate was also prepared by *N*-deacetylation and *N*-sulfonation of acharan sulfate (Fig. 7) (103,104). All these derivatives were analyzed for PASMC antiproliferative activity (Fig. 8) (105).

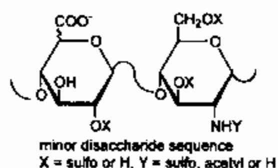
1. Heparin and Heparan Sulfate

Fully sulfonated heparin (Fig. 7) did not produce any greater growth inhibition of PASMC than did the parent heparin, indicating that native heparin already has

Heparin

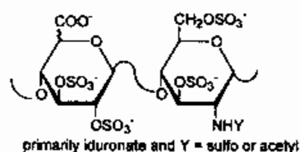


major disaccharide sequence



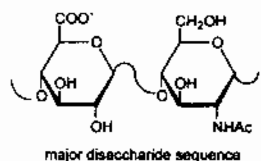
minor disaccharide sequence
X = sulfo or H, Y = sulfo, acetyl or H

Fully sulfated Heparin

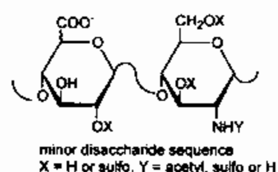


primarily iduronate and Y = sulfo or acetyl

Heparan sulfate

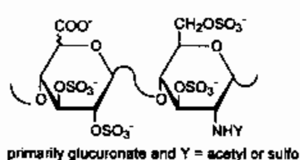


major disaccharide sequence



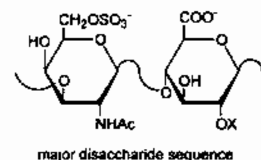
minor disaccharide sequence
X = H or sulfo, Y = acetyl, sulfo or H

Fully sulfated Heparan sulfate

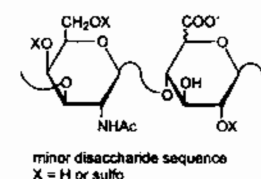


primarily glucuronate and Y = acetyl or sulfo

Chondroitin-6-*O*-sulfate
(Chondroitin sulfate C)

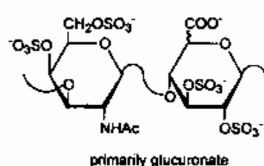


major disaccharide sequence



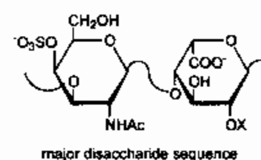
minor disaccharide sequence
X = H or sulfo

Fully sulfated Chondroitin sulfate

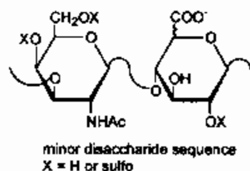


primarily glucuronate

Dermatan sulfate
(Chondroitin sulfate B)

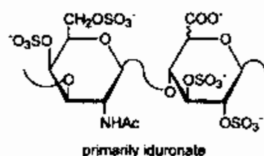


major disaccharide sequence



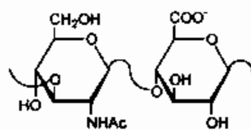
minor disaccharide sequence
X = H or sulfo

Fully sulfated Dermatan sulfate

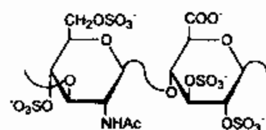


primarily iduronate

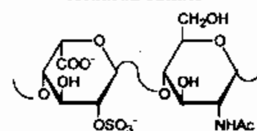
Hyaluronic acid



Fully sulfated Hyaluronic acid



Acharan sulfate



***N*-Sulfoacharan sulfate**

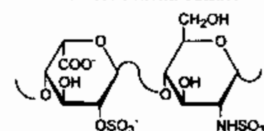


Figure 7 Major and variable sequences of original and fully sulfated glycosaminoglycans.

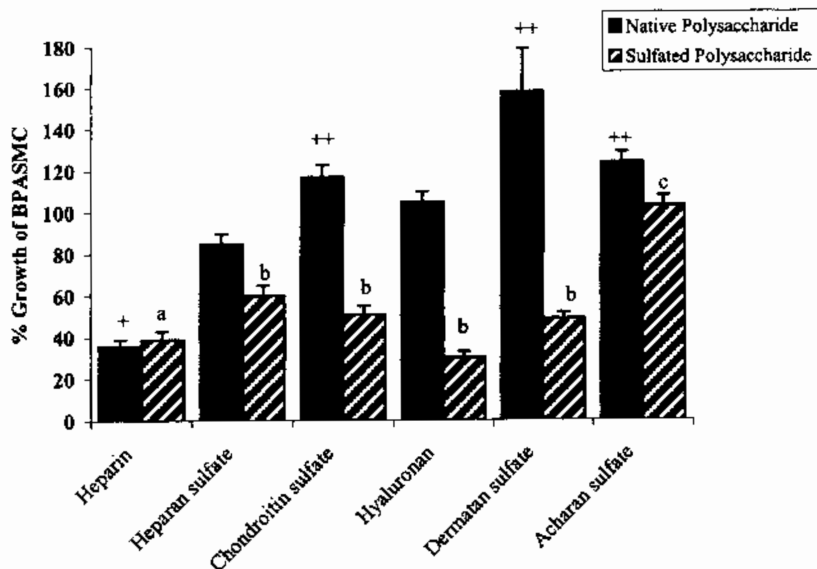


Figure 8 Effect of various polysaccharides on bovine pulmonary artery smooth muscle cells grown in media containing 10% fetal calf serum and either original (solid bars) or fully sulfonated glycosaminoglycan (striped bars) or acharan sulfate (solid bar) or N-sulfoacharan sulfate (striped bar). Samples are standardized to growth in media containing 10% serum without polysaccharide. [+] represents a significant reduction in cell growth as compared to the standard ($p < 0.05$); [++] represents a significant increase in cell growth as compared to the standard ($p < 0.05$); [a] represents a significant reduction in cell growth as compared to standard ($p > 0.05$); [b] represents a significant reduction in cell growth as compared to both the standard and the native polysaccharide ($p > 0.05$); [c] represents a significant reduction in cell growth as compared to native acharan sulfate ($p > 0.05$), but not to the standard (from reference 105, with permission).

the full complement of *N*- and *O*-sulfo groups necessary to produce maximum antiproliferative activity (Fig. 8) (105). However, fully sulfonated heparan sulfate containing both *N*-acetyl and *N*-sulfo substituents suppressed the growth of PASMC to a greater degree than the parent heparan sulfate, showing that heparan sulfate has insufficient *O*-sulfo groups for full antiproliferative potency.

2. Hyaluronan

The antiproliferative activity of native hyaluronan (Fig. 8) became strikingly significant after sulfonation and equaled that of native heparin (105). Since hyaluronan, like chondroitin and dermatan sulfates, has a 1→3 linkage between uronic

acid and hexosamine residues, whereas heparin and heparan sulfate have a 1→4 linkage, the linkages between its uronic acid and hexosamine residues (Fig. 7) do not seem to be critical for antiproliferative activity.

3. Acharan Sulfate

Neither acharan sulfate nor *N*-sulfoacharan sulfate had any antiproliferative activity. This result demonstrates that the presence of *O*-sulfo and *N*-sulfo groups on a glycosamine-iduronate backbone (Fig. 7) alone is insufficient to produce an antiproliferative activity (105). Indeed, the spatial positioning of these sulfo groups appears to play a major role in activity.

4. Chondroitin and Dermatan Sulfates

Full sulfonation of chondroitin and dermatan sulfates (Fig. 7) reversed their proliferative effect on PASMC and produced an antiproliferative effect similar to heparin (Fig. 8) (105). Both chondroitin and dermatan sulfate have a variable sequence with a low content of *O*-sulfo groups in their hexosamine residues. These data suggest that the presence of an enhanced level of *O*-sulfo groups on both hexosamine and uronate residues is necessary for antiproliferative activity. Since both chondroitin and dermatan sulfate GAGs contain *N*-acetylgalactosamine residues only, the above data also suggest that *N*-sulfoglucosamine residues in heparin can be replaced by a *N*-acetylgalactosamine residue. Further, since in chondroitin and dermatan sulfates all the hexosamine sugar residues contain *N*-acetyl groups, *N*-sulfo-substituted basic sugar residues appear not to be critical for antiproliferative activity.

F. Anticoagulant Activity

The anticoagulant activity of fully sulfonated heparin was significantly reduced in comparison to native heparin's anticoagulant activity. All the other sulfonated GAGs showed very little anticoagulation activity (105). This demonstrates that the structural determinants of heparin for its anticoagulant and antiproliferative activities are unrelated.

G. Minimum Oligosaccharide Size Requirement of Heparin GAG Chain

Previous studies on the size requirement for heparin's antiproliferative effect have shown that a tetrasaccharide was inactive. The smallest-size oligosaccharide that had some activity was a pentasaccharide (51). The dodecadisaccharide had the same antiproliferative activity as native heparin (53). We have found that a heparin oligosaccharide containing seven residues each of glucosamine and uronic

acid, i.e., a tetradecasaccharide (14-mer), is the minimum size oligosaccharide that is essential for antiproliferative potency as of native heparin (106). These differences of size requirement may be due to different cell types.

VII. Summary and Conclusion

In summary, the preceding studies on the effects of heparin and its derivatives on PASMC antiproliferative properties show:

1. 3-*O*-sulfo group substitution of glucosamine residues is not critical in whole heparin for antiproliferative activity.
2. Molecular weight of a given heparin (over the range examined) does not effect its potency as an antiproliferative agent.
3. Antiproliferative properties of heparin reside in the GAG chain, and not in the core protein.
4. A certain number of *O*-sulfo groups of heparin is essential for the full antiproliferative effect of heparin.
5. *N*-sulfo group on hexosamine residues is not critical for antiproliferative activity.
6. Hexosamine residues can be either glucosamine or galactosamine.
7. The anomeric linkage between uronic acid and hexosamine residues is not critical for antiproliferative activity.
8. Commercially available heparins have a varying degree of antiproliferative activity on PASMC.
9. Antiproliferative and anticoagulant activities reside in different domains of heparin.
10. The minimal oligosaccharide size requirements for antiproliferative activity is 14-mer.

The biosynthesis of heparin chains is initiated by the formation of *N*-acetylheparosan, a ($\rightarrow$4) β-D-GlcA (1$\rightarrow$4) α-D-GlcNAc $(1\rightarrow)_n$ polymer (Fig. 2) that is subsequently modified. The stepwise modification reactions are generally incomplete, in the sense that only a fraction of the potential substrate residues are utilized at each step. These processes therefore lead to sequence heterogeneity of heparin (Fig. 4). Functional properties of heparin and other proteoglycans depend heavily on their ability to bind receptors. Heparin binds with receptors in a specific manner. By virtue of this property, heparin possesses different types of biological activities which derive from different structural domains of the molecule. An increase in antiproliferative activity of fully sulfonated heparan sulfate, dermatan sulfate, chondroitin sulfate, and hyaluronan shows a potential for the development of one of these derivatives as a therapeutic agent for treatment of vascular remodeling in the near future.

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