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REVIEW

Dermatan Sulfate as a Potential Therapeutic Agent

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Abstract—1. Dermatan sulfate is a linear, sulfated polysaccharide and is a glycosaminoglycan component of several important proteoglycans. This minireview discusses the biosynthesis, structure and biological function of dermatan sulfate proteoglycans.

2. Dermatan sulfate and its derivatives are being investigated as a new class of anticoagulant and antithrombotic agents.

3. The preparation, chemistry and structure–activity relationship of dermatan sulfate is described.

4. Dermatan sulfate, low molecular weight dermatan sulfate and glycosaminoglycan mixtures containing dermatan sulfate have been used clinically.

5. The future prospects of these agents and other new, potentially useful dermatan sulfate based therapeutics are discussed.

Key Words: Dermatan sulfate, heparin cofactor II, antithrombotic, clinical use, structure-activity relationship, glycosaminoglycan

INTRODUCTION

Dermatan sulfate (DS) is a member of a family of structurally complex, sulfated, linear polysaccharides called glycosaminoglycans. The other members of this family of molecules are heparin, heparan sulfate, chondroitin sulfates and hyaluronic acid. DS and chondroitin sulfates are structurally similar and make up a subfamily of glycosaminoglycans called galactosaminoglycans. Glycosaminoglycans are often found attached to a protein core resulting in a macromolecule called a proteoglycan. These proteoglycans localize on cell surfaces and in the extracellular matrix (Hardingham and Fosang, 1992). There they function in the important role of cell–cell interaction, binding a variety of biologically important proteins and localizing these at the cell surface (Templeton, 1992; Hardingham and Fosang, 1992). Glycosaminoglycan DS has been used as an experimental therapeutic agent to modulate a variety of these biological processes.

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STRUCTURE AND BIOSYNTHESIS

DS is an important glycosaminoglycan found in a wide variety of tissues in virtually all animals. DS is a micro-heterogeneous, polydisperse, linear copolymer of *N*-acetyl-D-galactopyranose (D-GalpNAc), L-idopyranosyluronic acid (L-IdoAp) and D-glucopyranosyluronic acid (D-GlcAp) with *O*-sulfo groups most commonly found on the 4-position of D-GalpNAc residues and occasionally on the 6-position of D-GalpNAc and the 2-position of L-IdoAp residues. DS, sometimes referred to as chondroitin sulfate B, differs from chondroitin sulfate A and chondroitin sulfate C, by the presence of L-IdoAp in its structure (Fig. 1).

The biosynthesis of the DS glycosaminoglycan chain occurs on a protein core resulting in the formation of a proteoglycan. Several DS core proteins have been identified (Cöster, 1991; Kresse, Hausser and Schönherr, 1993). These include large proteoglycans with up to 25–30 DS polysaccharide chains and small proteoglycans such as decorin and biglycan, with 1 and 2 DS polysaccharide chains, respectively. DS biosynthesis is a multi-step process. First, the core protein is synthesized after which one

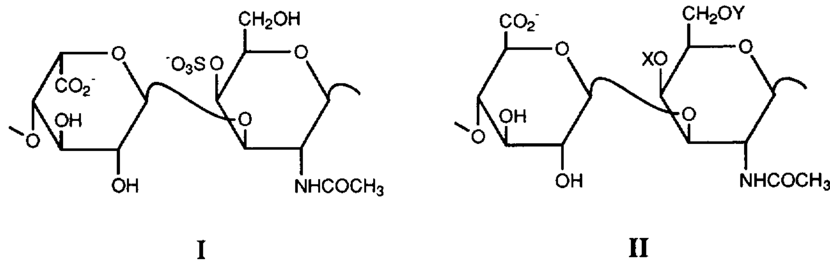


Fig. 1. The major disac charide repeating units of dermatan sulfate (structures I and II) and chondroitin sulfates (structure II). In chondroitin sulfate A, $X = SO_3^-$ and $Y = H$, while in chondroitin sulfate C, $X = H$ and $Y = SO_3^-$.

or several protein–polysaccharide linkage regions are formed, initiating the synthesis of the polysaccharide chain through the sequential addition of UDP activated D-GlcAp and D-GalpNAc residues. Concurrent with or immediately following chain elongation, the polysaccharide is enzymatically modified by C-5 epimerization of D-GlcAp to L-IdoAp and *O*-sulfated at the 4-position of D-GalpNAc (Fig. 1, I) and to a lesser extent at the 2-position of L-IdoAp and the 6-position of D-GalpNAc (Malmström, 1984). The C-5 epimerization and *O*-sulfation reactions are typically incomplete leaving unmodified regions of →4)GlcAp(1→3)GalNAc(1→ sequences. This incomplete modification gives rise to microheterogeneity in the DS polymer (Fig. 1, I & II).

The synthesis of decorin and biglycan core proteins are under the control of platelet-derived growth factor (PDGF) as well as transforming growth factor-β (TGF-β) (Border *et al.*, 1990; Schönherr *et al.*, 1993). In cultured arterial smooth muscle cells, transforming growth factor-β increases biglycan synthesis more than three-fold by doubling of the steady-state level of biglycan mRNA transcripts. Transforming growth factor-β and platelet-derived growth factor increase the molecular size of the glycosaminoglycan chain in both decorin and biglycan. Although platelet-derived growth factor does not affect the steady-state level of decorin mRNA, addition of platelet-derived growth factor to cultured cells increases the ratio of 6-sulfation to 4-sulfation. Thus, the biosynthesis of the protein core molecule is transcriptionally regulated and the modifications to the glycosaminoglycan chain structure are influenced by specific growth factors.

LOCALIZATION AND BIOLOGY OF DERMATAN SULFATE

Proteoglycans are found in the pericellular and extracellular matrix of most eukaryotic cells. DS proteoglycans are found in fibrous connective tissues such as skin, tendon and sclera as well as skeletal muscle, blood vessels, bone and cartilage. The DS protein core molecule is rich in leucine (Kresse *et al.*, 1993) and is involved in both protein–protein and protein–lipid interactions. Decorin DS proteoglycan binds collagen types I and II playing an important role in the organization of collagen fibrils. Indeed, the specific blend of proteoglycan and collagen determines the elasticity and transparency properties of the tissue (Cöster, 1991). Biglycan DS proteoglycan does not bind collagen types I and II, is found in cell surface and pericellular environments and its function is not well understood. Although the protein core and glycosaminoglycan structure of decorin and biglycan are similar, their expression and localization is quite different possibly giving rise to their different biological roles (Hardingham and Fosang, 1992).

DS proteoglycans, biglycan and decorin may play a role in regulating the extravascular activities of thrombin. In solution, biglycan, decorin and their glycosaminoglycan chains accelerate heparin cofactor II (a plasma serine protease inhibitor) inhibition of thrombin (factor IIa, a plasma serine protease) (Fig. 2). Both biglycan and decorin exhibit the same activity when bound to type V collagen. This observation suggests that one function of these DS proteoglycans is to provide a thromboresistant extravascular surface (Whinna *et al.*, 1993). Thrombomodulin is a proteoglycan found on the luminal surface of the vascular endothelium and on the underlying smooth muscle cells. It contains a single chondroitin/dermatan sulfate glycosaminoglycan chain. Thrombomodulin binds thrombin, presumably through interaction with both its protein core and its glycosaminoglycan chain (Bourin and Lindahl, 1993). Once bound, thrombin can act on protein C to form protein Ca, an activated serine protease, which inactivates factors Va and VIIIa, preventing the generation of factor Xa and thrombin, thus inhibiting coagulation (Fig. 3). Thrombin bound to thrombomodulin is also sensitive to inhibition by antithrombin III.

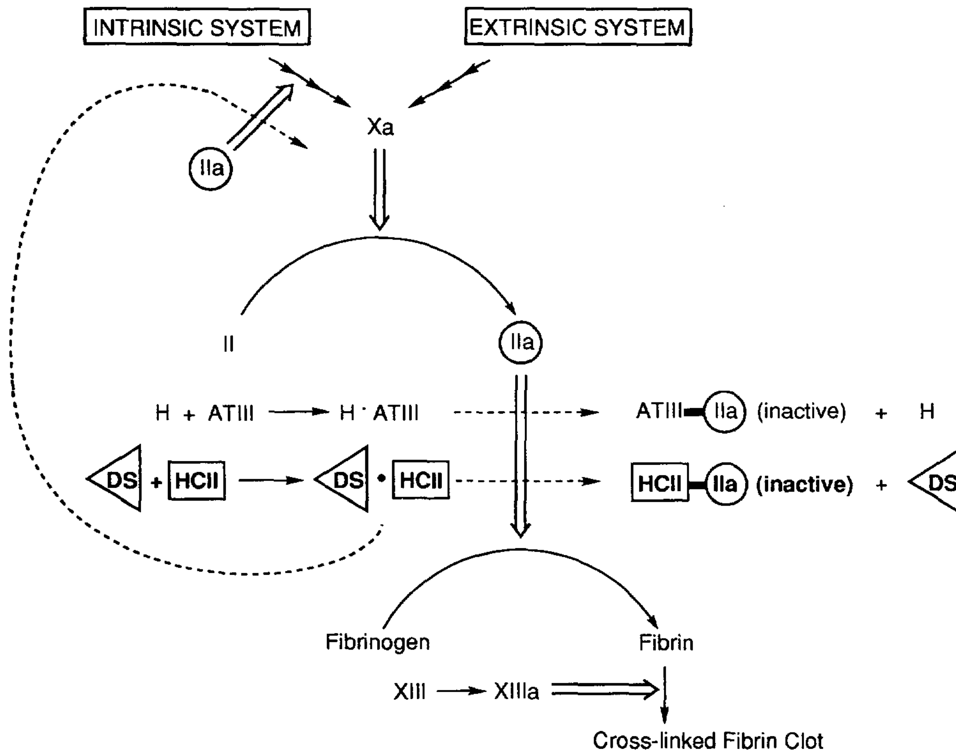


Fig. 2. Mechanism for DS catalyzed heparin cofactor II inhibition of thrombin. DS binds to heparin cofactor II (HCII), interaction of thrombin (IIa) with this complex causes its inactivation. DS is released to catalyze additional reactions. Thrombin (IIa) can activate the factors of the intrinsic pathway and also acts to convert fibrinogen to fibrin which is then cross-linked to form an insoluble clot. Thrombin (IIa) is also inhibited by antithrombin III (ATIII) in the presence of heparin (H).

Many of the biological activities of DS proteoglycans are associated with their glycosaminoglycan chains (Whinna *et al.*, 1993; Bourin and Lindahl, 1993). Glycosaminoglycan DS has important anticoagulant (Linhardt *et al.*, 1991a, b; Andrew *et al.*, 1992) and antithrombotic (Pangrazzi and Gianese, 1987) activities. The mechanisms for these activities are complex involving soluble plasma proteins, the endothelial cells lining the vessel wall (Buchanan *et al.*, 1993), subendothelial smooth muscle cells, platelets and the fibrinolytic pathway (Benayan *et al.*, 1994).

The glycosaminoglycan chains of decorin, biglycan and thrombomodulin can act as anticoagulants by inhibiting thrombin, either directly through heparin cofactor II or antithrombin III, or indirectly through protein C activation (Fig. 3) (Whinna *et al.*, 1993; Bourin and Lindahl, 1993). Since these proteoglycans are found on both the luminal surface and subluminal surface they provide a localized anticoagulant affect affording thromboresistant surfaces at sites in both the intact and damaged vessel.

Dermatan sulfate also acts as an antithrombotic agent by inhibiting the thrombin induced aggregation of platelets and may activate the fibrinolytic pathway by causing the release of tissue plasminogen activator (tPA) (Pangrazzi and Gianese, 1987).

The most thoroughly studied activity associated with DS is its acceleration of heparin cofactor II mediated inhibition of thrombin (Tollefsen, 1992). The discovery of this second circulating thrombin inhibitor has focused attention from heparin (found in mast cells) to endogenous glycosaminoglycans (found in the vasculature) such as dermatan sulfate and heparan sulfate. The mechanism of heparin cofactor II mediated anticoagulation is shown in Fig. 2.

DS may play a role in lipid metabolism. DS, like other GAGs, bind and promote the release of lipoprotein lipase from endothelial cells into the circulation in an active form (Pangrazzi and Gianese, 1987). The composition of the endothelial cell surface proteoglycans heparan sulfate and DS are found to change during atherogenesis (Edwards *et al.*, 1990).

PREPARATION OF DERMATAN SULFATE

Glycosaminoglycan DS is routinely prepared commercially from porcine and bovine intestinal mucosa or porcine skin. Typically, tissue is treated

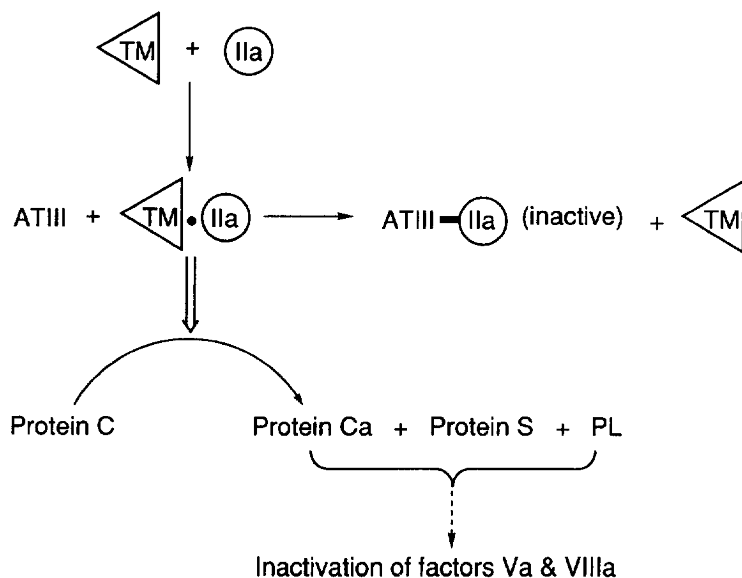


Fig. 3. Mechanism of anticoagulation by thrombomodulin (TM) proteoglycan. TM catalyzes antithrombin III (ATIII) inhibition of thrombin (IIa). TM·IIa complex also catalyzes the activation of protein C. In the presence of protein S and phospholipids (PL) protein Ca inactivates factors VIIIa and Va.

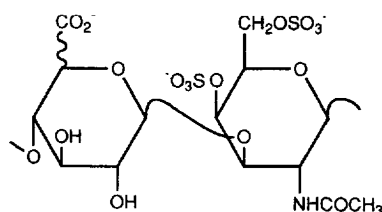
extensively with protease and crude DS is recovered by precipitation. A detailed procedure has been outlined by Rodén *et al.* (1972) where, after protease treatment of tissue using papain, the digest is filtered through Celite and the filtrate precipitated with cetylpyridinium chloride. The precipitate is then dissolved in a sodium chloride solution reprecipitated with ethanol and recovered by centrifugation. The crude glycosaminoglycan precipitate is then dissolved and precipitated from alkaline Benedict's solution. Precipitated DS is recovered, dissolved in dilute acetic acid and copper is removed using protonic Dowex chromatography. Strong anion-exchange chromatography is often used to further purify DS.

CHEMISTRY OF DERMATAN SULFATE

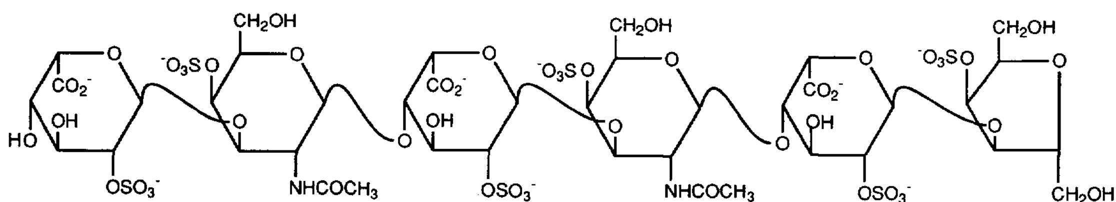
DS is a copolymer of D-glucuronic acid or L-iduronic acid and *N*-acetyl-D-galactosamine. The molecular weight of DS ranges from 12 to 45 kDa with an average molecular weight ranging from 20 to 30 kDa. The ratio of D-GlcAp to L-IdoAp varies depending on the tissue source (Linhardt *et al.*, 1991a) as well as the method of preparation (Rodén *et al.*, 1972). The L-IdoAp content of DS polymers may range from 1 to over 90% (Kresse *et al.*, 1993). Other differences in the primary structure of DS include sulfation at the 4- and 6-positions of D-GalpNAc and the 2-position of L-IdoAp and D-GlcAp residues. DS contains approximately 1 sulfate group per disaccharide unit (Bourin and Lindahl, 1993).

STRUCTURE–ACTIVITY RELATIONSHIP OF DERMATAN SULFATE

The structure–activity relationships of dermatan sulfate's anticoagulant and antithrombotic activities, particularly those associated with its heparin cofactor II mediated inhibition of thrombin, have been studied. When DS is fractionated by gel permeation chromatography, to obtain fractions of average molecular weight 10,000–60,000, little difference *in vitro* heparin cofactor II mediated antithrombin activity is observed (Linhardt *et al.*, 1991b). But when intact DS is partially depolymerized, a minimum chain size of 12–14 saccharide residues is found to be required for maintenance of heparin cofactor II mediated antithrombin activity (Tollefsen *et al.*, 1986; Linhardt *et al.*, 1994). Fractionation of intact DS chains by anion-exchange chromatography affords fractions that differ 10-fold in heparin cofactor II mediated antithrombin activity (Linhardt *et al.*, 1991b). The most highly sulfated chains, exhibiting the greatest activity, also are enriched in sequences of the structure →4)UAp(1→3)-β-D-GalpNAc4S6S(1→ (where UAp is either IdoAp or GlcAp) (Fig. 4, III). Mascellani *et al.* (1994) showed that base catalyzed removal of the 6-sulfate group from β-D-GalpNAc4S6S residues significantly reduces DS



III



IV

Fig. 4. The structure of a disaccharide III enriched in DS having high heparin cofactor II activity and the structure of the hexasaccharide IV that binds to heparin cofactor II.

catalyzed inhibition of thrombin by heparin cofactor II.

Maimone and Tollefsen (1990) identified a hexasaccharide as the smallest DS sequence still having the ability to bind heparin cofactor II with high affinity. In their study, DS was partially depolymerized using a controlled hydrazinolysis reaction followed by treatment with nitrous acid and borohydride, resulting in a 2,5-anhydro-D-talitol ($ATal_R$) residue at the reducing ends of the fragments. Controlled hydrazinolysis deacetylates a portion of the D-GalpNAc residues and nitrous acid treatment only cleaves the glycosidic linkages at these D-GalpN residues. The partially depolymerized DS was fractionated using gel permeation chromatography and further purified using heparin cofactor II–Sepharose affinity chromatography. The smallest fragments (hexasaccharide pool) that bound to heparin cofactor II were subjected to strong anion-exchange chromatography and one major component was identified. Exhaustive depolymerization of the high affinity hexasaccharide using the same hydrazinolysis and deamination method resulted in only one disaccharide, IdoAp2S–$ATal_R$4S. Thus, the deduced hexasaccharide has the sequence: IdoAp-2S–GalpNAc4S–IdoAp2S–GalpNAc4S–IdoAp2S–$ATal_R$4S (Fig. 4, IV).

This hexasaccharide stimulated inhibition of thrombin activity, was approximately 20-fold less active than unfractionated DS.

The results of structure–activity studies on the heparin cofactor II mediated antithrombin activity of DS pinpoints a minimum size of 12–14 saccharide units for significant activity (6 saccharide units for heparin cofactor II binding). The active DS chains appear to be enriched in the disulfated disaccharide sequences →4)UAp(1→3)GalpNAc4S6S(1→ or →4)IdoAp2S(1→3)GalpNAc4S(1→. The particular disulfated sequence observed in an active DS might be attributable to the species or tissue from which the DS is obtained.

The antithrombotic effect of DS is usually measured using two *in vivo* antithrombotic assays (Linhardt *et al.*, 1994). The Wessler stasis model, the most widely used, is considered to be a good screening model but is not physiological as blood is not flowing. The rat jugular clamping model may be more physiologic since it measures blood flow through a damaged vessel and thus, more closely mimics human vascular disease. Studies examining the *in vivo* antithrombotic activity of DS, using both models, suggest that antithrombotic activity correlates poorly with *in vitro* heparin cofactor II mediated antithrombin activity (Mascellani *et al.*, 1989; Linhardt *et al.*, 1991a, 1994). DS samples prepared identically from bovine and porcine intestinal mucosa having both high and low *in vivo* antithrombotic activity showed no correlatable differences in heparin cofactor II mediated antithrombin activities. DS samples treated with periodate and reduced with borohydride (opening the unsulfated UAp residues without

fragmenting the DS chain) affords material with substantial antithrombotic activity but virtually no heparin cofactor II mediated antithrombin activity (Mascellani *et al.*, 1989). The antithrombotic activity of DS samples seems to correlate best with global assays such as the activated partial thromboplastin time (Linhardt *et al.*, 1991a). Chemical depolymerization of DS, however, shows that approximately the same minimum chain size (12–14 sugar residues) is required for both *in vivo* antithrombotic and *in vitro* heparin cofactor II mediated antithrombin activities (Tollefsen *et al.*, 1986; Linhardt *et al.*, 1994).

Several conclusions can be drawn from these studies. While heparin cofactor II mediated antithrombin activity may play a role in dermatan sulfate's antithrombotic activity, other less understood biochemical mechanisms are also involved. The antithrombotic activity of DS can be correlated with an increased level of sulfation, an increased content of IdoAp residues (Linhardt *et al.*, 1994) and a required minimum molecular weight. A high content of IdoAp also results in an increased flexibility of DS, enhancing its ability to self-associate and possibly to interact with plasma proteins (Casu, 1991). More study is certainly needed to more fully understand dermatan sulfate's complex biological activity and the optimum DS structure required for elaboration of this activity.

CLINICAL USE OF DERMATAN SULFATE

Although the anticoagulant activity of DS is significantly less than heparin, its venous antithrombotic activity appears to be significantly higher (Desnoyers *et al.*, 1989). Thus, the hemorrhagic properties of DS are greatly reduced when compared to heparin. This has made DS an interesting target in developing new therapeutic agents for the prevention of thrombosis.

The anticoagulant effect of DS is very difficult to measure using traditional clotting tests such as activated partial thromboplastin time and thrombin time assays since DS is approximately 70-fold less potent than heparin on a per weight basis (Sié *et al.*, 1991). Thus, nonclotting assays have been developed to measure plasma DS concentrations and are reviewed by Sié *et al.* (1991). Methods to detect DS in plasma include a competitive binding assay which measures the plasma DS concentration and a chromogenic assay for DS which measures the ability of plasma DS to inhibit thrombin through heparin cofactor II (Dupouy *et al.*, 1988).

Boneu *et al.* (1992) reported that DS has two major pharmacokinetic problems, a relatively short half-life and low bioavailability, when compared to heparin delivered by subcutaneous or intramuscular routes. DS, however, does not bind to the endothelium and thus gives simpler pharmacokinetics than those observed for heparin.

The use of DS in clinical trials has been extremely limited (Bergquist *et al.*, 1992). A pharmaceutical preparation of DS, MF 701, has been characterized and is reviewed by Gianese and Lucchelli (1991). MF 701 has been used in healthy volunteers and patients under treatment for renal failure by dialysis, for venous thromboembolism, disseminated intravascular coagulation syndrome and arterial atherothombotic diseases. In all cases (greater than 300 patients) no serious bleeding complications have occurred with doses ranging to 2 mg/kg. More recently, MF 701 has been used to prevent deep vein thrombosis in hip fracture patients. Imbimbo *et al.* (1994) reported that a minimum DS plasma concentration of 9 μg/ml was necessary to optimize efficacy. In a separate study (Confracesco *et al.*, 1994), the efficacy of moderate intraveneous doses of MF 701 was compared to heparin for the treatment of disseminated intravascular coagulation in acute leukemia patients. DS (infusion rate of 0.3 mg/kg/hr) was as effective as heparin (8.5 mg/kg/hr) in preventing deep vein thrombosis. Moreover, new bleeding complications were less frequent with MF 701 DS than heparin and no patient experienced thromboembolic complications.

DERMATAN SULFATE DERIVATIVES

The high molecular weight of DS inhibits its absorption when administered subcutaneously or intramuscularly. Indeed, as the DS polymer size increases, the bioavailability decreases (Boneu *et al.*, 1992). Thus low molecular weight fractions of DS have been prepared in attempts to further improve their bioavailability and pharmacokinetic properties (Saivin *et al.*, 1992; Boneu *et al.*, 1992). Low molecular weight DS may also have improved antithrombotic activity, reflecting an increase in the therapeutic index.

Low molecular weight DS is prepared by controlled depolymerization of DS using hydrogen peroxide and a copper (II) salt catalyst (Volpi *et al.*, 1992; Linhardt *et al.*, 1994), or by using periodate oxidation followed by borohydride reduction and acid hydrolysis (Mascellani *et al.*, 1989). The partially depolymerized DS is then fractionated using gel permeation (Linhardt *et al.*, 1994), strong anion-exchange (Mascellani *et al.*, 1989) or affinity (Griffith and Marbet, 1983; Tollefsen, 1992) chromatography. The molecular weight of a fractionated DS sample can be determined by a variety of methods such as gel permeation chromatography, viscometry and poly-

acrylamide gel electrophoresis, using molecular weight standards (Linhardt *et al.*, 1991a, b, 1994).

Oversulfated DS derivatives have been prepared by the sulfation of DS with sulfur trioxide–triethylamine complex (Maaroufie *et al.*, 1990). These DS derivatives contained 2–3 sulfate groups per disaccharide unit as compared to 1 sulfate group per disaccharide in DS. The anticoagulant activity increased with increased sulfation as measured by all assays (Maaroufi *et al.*, 1990). The pharmacokinetics of oversulfated DS became more complex, exhibiting a dose dependent half-life, suggesting that these derivatives behaved more like heparin than DS (Boneu *et al.*, 1992). This increased complexity probably results from the binding of oversulfated DS to the endothelium. While oversulfated DS shows enhanced antithrombotic activity they show a concomitant increase in hemorrhagic complications (Van Ryn-McKenna *et al.*, 1989).

GLYCOSAMINOGLYCAN MIXTURES CONTAINING DERMATAN SULFATE

The rationale for using a GAG mixture containing DS is to increase the therapeutic efficiency while reducing risks of hemorrhagic side-effects. Cadroy *et al.* (1988) has shown that a mixture of standard heparin and DS (doses of 10 mg/kg and 25–35 mg/kg, respectively) was more efficient in thrombus prevention in rabbits than standard heparin or DS alone at the same dosages. Lomoparan (Org 10172) contains dermatan sulfate, heparan sulfate and small amounts of chondroitin sulfate. Lomoparan was shown to be at least as effective as low dose heparin or warfarin without an increased risk of bleeding complications. The clinical use of Lomoparan has been reviewed (Berquist *et al.*, 1992; Gordon *et al.*, 1990). Sulodexide, a mixture of heparin and dermatan sulfate, was found to be more effective than heparin alone in thrombus inhibition at reduced dosages (Sulodexide and heparin 260 μg/kg and 800 μg/kg, respectively) (Buchanan *et al.*, 1994). In DS and heparin mixtures, the synergistic effect of both antithrombin III and heparin cofactor II catalyzed inhibition of thrombin may have a greater therapeutic value.

FUTURE PROSPECTS FOR DERMATAN SULFATE BASED THERAPEUTICS

There are a number of future prospects for DS based therapeutics. Standard DS might be introduced as an antithrombotic agent following successful outcome of clinical trials. Low molecular weight DS preparations might have the same potential advantages over DS as low molecular weight heparins have over heparin. Low molecular weight DS agents would require extensive clinical evaluation before they could be used as pharmaceuticals. DS as a component of glycosaminoglycan mixtures may have utility as new antithrombotic agents. The major problem with all of these DS based agents is that they are polydisperse mixtures. This complicates their manufacture, use and analysis; in the case of the related low molecular weight heparins, this microheterogeneity and polydispersity has delayed their approval by the United States Food and Drug Administration.

Current pharmaceutical research is focused on the preparation of a new generation of glycosaminoglycan mimetics. These are small sulfated or sulfonated molecules prepared synthetically that mimic the activity of glycosaminoglycans such as DS by tightly binding to the proteins with which they interact (i.e. heparin cofactor II). The major advantage of these agents is that they are homogenous chemical entities while their major disadvantages is their potential toxicity. Clinical studies will be essential to determine whether such compounds represent a successful new class of therapeutic agents.

One of the newest applications for DS is in the preparation of medical devices and artificial tissues. Stone (1989) has patented a prosthetic meniscus to be used as an knee implant which acts as a scaffold for the regrowth of native meniscal tissue. The material is composed of collagen fibrils interspersed with GAG molecules including DS. DS has been more recently shown to be useful in the development of artificial tissues. Miwa and Matsuda (1994) have designed small arterial prostheses composed of a microporous polyurethane tube coated with a gel containing a mixture of type I collagen and DS. This gel promoted the adhesion and growth of endothelial cells, and reduced platelet adhesion *in vitro*. The authors reported that the grafts seeded with endothelial cells were highly antithrombotic when implanted into the carotid arteries of dogs. New applications of DS in implantation, tissue culturing and transplantation are on the horizon.

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