Dermatan Sulphate as an Antithrombotic Drug

Giuseppe G. Nenci

Medicina Interna e Cardiovascolare, Università di Perugia, Italy

Key Words

Dermatan sulphate, glycosaminoglycans, venous thromboembolism, haemodialysis, heparin-induced thrombocytopenia.

Abstract

Dermatan sulphate (DS) is a glycosaminoglycan which selectively catalyzes the inactivation of thrombin by Heparin Cofactor II without interacting with Antithrombin III. DS does not interact with other coagulation factors and, unlike heparin, is able to inactivate thrombin bound to fibrin or to the surface of an injured vessel. Efficacy and safety of DS have been validated in studies on thromboprophylaxis and on the anticoagulation for hemodialysis. Studies on thromboprophylaxis have been performed in "medical" patients as well as in general, orthopedic and oncological surgery. In this last setting, DS proved to be more efficacious than heparin, in the absence of excess bleeding. No statistically significant differences were observed between DS and heparin in hemodialysis. A low-molecular-weight DS, which shows a higher bioavailability after s.c. administration, has been tested in pilot studies on the treatment of venous thromboembolism with encouraging results. Two DS-containing compounds, sulodexide

and, particularly, mesoglycan, have been clinically studied in a number of trials and found to be effective in the treatment of venous and arterial leg diseases.

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Dermatan Sulphate (DS) belongs to the family of glycosaminoglycans (GAGs) which are naturally occurring mucopolysaccharides with varying degrees of sulfation.

DS is largely distributed in connective tissues of mammals and other vertebrates, including the vessel wall. Like heparin, DS is extracted from porcine intestinal mucosa.

Other compounds, consisting of mixtures of different GAGs, are only available for clinical use in selected countries (for example mesoglycan and danaparoid, both of which contain heparin sulfate, dermatan sulfate and smaller fraction of other GAGs; or sulodexide, which is a fast-moving heparin derivative associated with a lesser amount of DS).

DS selectively catalyzes the inactivation of thrombin by Heparin Cofactor II (HCII) with an allosteric mechanism, without interacting with antithrombin III (AT). HCII, in turn, does not interact with coagulation factors other than thrombin. Therefore, DS differs from heparin in that it acts on the coagulation system as a selective thrombin inhibitor and is able to inactivate thrombin bound to fibrin or the surface of an injured vessel more effectively than heparin (Bendáyan et al) [1]. A hexasaccharide sequence which is responsible for DS binding to HCII has been described by Maimone and Tollefsen [2]. Other pharmacological

properties of DS having potential clinical relevance are its reduced susceptibility to neutralization by plasma proteins (Cosmi et al) [3, 4], its linear pharmacokinetics and its long half-life after intramuscular administration (Saivin et al, unpublished data), and its low experimental toxicity.

The only DS preparation commercially available is produced by Mediolanum Farmaceutici, Italy, according to a patented process which ensures a product as similar as possible to the natural one.

The active substance produced until 1994 and used in the early phase of DS development, had a typical mean molecular weight of 27 kD; DS produced since then has a typical molecular weight of 22 kD, with 42% below 20 kD. Anti-factor Xa activity is below 2U/mg and anti-thrombin activity is 40-60U/mg (Standard Units DS).

A low molecular weight (LMW) DS has been prepared by limited radical depolymerization of natural DS by Opocrin and studied by Alfa-Wasserman, Italy. This product is endowed with the same activities of natural DS, however it shows also an AT-mediated inhibition of factor Xa, apparently not explained by the associated low molecular weight heparin (LMWH) component. MW is about 5,500 with slight heparin contamination (about 5U/mg a-Xa and 89U/mg a-IIa activity, chromogenic assay).

Both preparations induce a slight fibrinolysis due to the release of tissue plasminogen activator (t-PA) by the vascular endothelium (Abbadini et al; Legnani et al) [5, 6]

Activity on the coagulation and fibrinolysis system

Thrombin inhibition in purified systems by DS was 6.5% of that of unfractionated heparin in the hands of Toulon et al [7].

Significant differences were found by Iorio et al [8] in the relative sensitivities of various commercial APTT reagents to DS. The DS concentration required to double APTT in human plasma varied from 37 to 60 mg/ml when determined with five different reagents. APTT-ellagic acid (Instrumentation Laboratories) was found to be the most sensitive reagent among those tested.

After addition of DS to human plasma, a measurable anti-factor Xa activity was detected by the Yin and Wessler's coagulation method which was however consistently absent using the specific chromogenic substrate assay (Toulon et al) [7]. Therefore, the activity detected by the former method was considered to be a non-specific consequence of thrombin neutralization by LMW-DS.

The dose-dependency of heparin pharmacokinetics and the marked inter-individual variability of its anticoagulant effects are partly due to binding and neutralization of heparin by plasma and platelet-secreted proteins (Hirsh) [9] and particularly, acute-phase proteins.

Cosmi et al [4] investigated the recovery of DS and unfractionated heparin in normal human plasma samples or samples from patients with venous thromboembolism. Mean plasma recovery of heparin was significantly lower in patient plasma

(74%) than in normal plasma (101%). In contrast, there was total recovery of DS in both patient and normal plasma.

Thrombin bound to a fibrin clot remains enzymatically active and is therefore considered responsible for the growth of fibrin-rich thrombi (Weitz et al) [10].

The protection of fibrin-bound thrombin by heparin reflects heparin-mediated bridging of thrombin to fibrin; this ternary complex limits accessibility of heparin-catalyzed inhibitors to thrombin, while DS binds to thrombin but not to fibrin. Therefore DS is a more potent inhibitor of clot-bound thrombin than heparin (Liaw et al) [11].

The effects on the fibrinolytic system have been studied by several authors. Abbadini et al [5] showed that DS induced a significant release of t-PA in the perfusate while u-PA remained unchanged. UFH, tested in the same model at comparable concentration, was inactive. This represents an effect of DS on the vessel wall, since the compound was devoid of intrinsic fibrinolytic effect (on fibrin plates) and was incapable of potentiating t-PA activity in "vitro".

Antithrombotic and hemorrhagic activity

The antithrombotic activity of DS has been documented in several models of arterial or venous thrombosis. In the rat DS did not induce bleeding up to the dose corresponding to the maximal antithrombotic effect observed. On the contrary, UFH induced a prolongation of bleeding time with a sharp increase starting at 0.5 mg/kg (Maggi et al) [12].

In the rabbit, at 20 times the antithrombotic dose, blood loss was significantly increased by UFH but not by DS. The toxicology data which have been obtained following overdosage of animals by the intravenous and intramuscular routes afford a satisfactory safety margin with respect to the clinically relevant doses proposed for DS.

Pharmacokinetics and safety in human volunteers

After a single intravenous bolus of "natural" DS plasma levels decline mono-exponentially according to a substantially linear behaviour with a short half-life (less than 1 hour). The distribution volume is close to the plasma volume (Boneu et al, unpublished data).

The subcutaneous route of administration is impractical because of the rather high volumes to be administered. After single intramuscular doses of 100-300 mg, plasma concentrations were 0.8-1.4 µg/ml and elimination T₂ markedly more prolonged (6.2-9.7h) than after i.v. administration. After the dose of 300 mg, detectable plasma concentration persisted for 24h (Dawes et al) [13].

DS currently produced by Mediolanum Farmaceutici showed a bioavailability of 53.7% after a single i.m. dose of 300 mg and 79.1% after repeated (once daily) dosing. APTT (Organon Teknika) ratio reached the value of 1.6 at the end of an 8 h infu-

sion of 300 mg DS, and of 1.0-1.4 after repeated i.m. daily injections of the same dose. TCT (human thrombin) ratio fluctuated between 1.1 and 3.3 (Saivin et al, unpublished data).

LMW-DS behaved differently for some aspects. After an i.v. bolus of 400 or 800 mg, volumes of distribution were higher than plasma volume and T/2 was about 1-2 hours; APTT and TCT ratios showed short lived elevations (1.5-2.1 and more than 20, respectively) and an unexpected inhibition of FXa reached values of 0.95-1.27 "heparin" IU/ml (Dettori et al)[14]. FXa inhibition has been confirmed by other studies, even after subcutaneous (Harenberg et al) [15] or intramuscular (Legnani et al) [6] administration. The anti-Xa effect seems not to be attributable to contamination by traces of LMWHs, because only a negligible anti-Xa activity was seen after addition of LMW-DS to plasma "in vitro". It is presumed that some other GAG endowed with anti-Xa activity, is released from the vessel wall after the administration of this drug (Legnani et al)[6].

Clinical efficacy and safety

The efficacy and safety of DS have been tested in the prevention of venous thromboembolism (VTE) and in the anticoagulation for hemodialysis.

In addition, pilot studies have been conducted in the treatment of acute ischemic stroke, of disseminated intravascular coagulation in acute leukemia, of heparin-induced thrombocytopenia-thrombosis (HIT) and of VTE.

In thromboprophylaxis studies DS was administered by the intramuscular route, due to the need to inject volumes up to 3 ml, which are too large for subcutaneous injection. For the same reason DS doses >400 mg/day were subdivided in two daily injections, even though a once daily regimen would have been justified by the pharmacokinetic data. In studies concerning the other indications DS was administered by the intravenous route.

Early thromboprophylaxis studies (until 1995) used the "natural" DS preparation while later studies used the current Mediolanum-DS.

In these studies, the control group received calcium-heparin. Placebo injections were used when no gold-standard prophylaxis existed at that time. In the general surgery study (Prandoni et al) [16], the prophylactic efficacy of DS at the intramuscular dose of 100 mg/day appeared to be similar to that of subcutaneous low-dose heparin. The absolute incidence of DVT was probably underestimated in patients surveilled with impedance plethysmography (IPG) alone or ultrasonography, due to the limited sensitivity of these tests. In effect, patients undergoing IPG plus radio-fibrinogen uptake test had a higher DVT rate than those (the majority) undergoing IPG alone.

The hemorrhagic potential of DS was significantly lower than that of heparin prophylaxis in general surgery patients. The advantage of DS was clinically important, as indicated by the need for fewer reoperations due to bleeding (1 vs 10, $p=0.01$) and fewer post-operative transfusions (16 vs 29, $p=0.04$).

The results obtained, in an internal medicine trial (Zacá and Puddu, unpublished data), probably reflect the low incidence of DVT in the patient sample examined, combined with a low sensitivity of the diagnostic test used for surveillance (Doppler sonography). Therefore, no firm conclusion could be drawn on the efficacy of DS in this indication. On the other hand, the double-blind comparison with placebo in this trial allowed for an accurate assessment of safety of DS. A daily intramuscular dose of 100 mg DS did not cause any increase in the risk of bleeding (as assessed both clinically and on the basis of hematocrit and hemoglobin levels). Patients with acute MI or stroke were not included in this trial.

One small feasibility study followed by a placebo-controlled multicenter trial on 95 patients (Dehen et al, unpublished data) investigated efficacy and safety of DS in continuous infusion vs Dalteparin 2500U s.c. o.i.d., for the treatment of acute ischemic stroke. There were no differences in the neurological outcome.

The effects of DS on laboratory and clinical signs of disseminated intravascular coagulation (DIC) were studied in ten patients with acute leukaemia treated with DS as a continuous intravenous infusion (7.2 mg/kg/day, $n=5$) or with heparin (200U/kg/day, $n=5$), until the remission of laboratory signs of DIC (9-18 days) (Cofrancesco et al) [17]. Regression of the laboratory signs of DIC was obtained in all but one patient of the heparin group.

DS has been used by us for the treatment of HIT in five patients with favourable outcome in four of them (Taliani et al) [18]. A low platelet count persisted in a patient with cross-reactivity to DS. A larger study is now under way.

The studies conducted in orthopedic surgery [19, 20] explored different intramuscular regimens of DS, using bilateral contrast venography to detect DVT. The results were consistent in indicating that prevention of venous thromboembolism was obtained in these patients at the dose of 600 mg/day DS, the absolute incidence of residual DVT with the effective dose remained higher than desirable (approximately 35%). However, if reference is made to published venographic studies on heparin, the protective effect of DS against DVT appeared to be similar to that of heparin in elective hip surgery, and superior in hip fracture (Clagett et al). [21]. DS appeared to be virtually devoid of hemorrhagic effects up to the effective dose of 600 mg/day, as indicated by the absence of significant differences with placebo (Agnelli et al) [19], or between increasing doses of DS (Cohen et al) [20] in all of the pertinent clinical, transfusion and hematological indexes. The results of hip fracture studies indicated that prolonged preoperative treatment with DS neither increases intraoperative bleeding, nor compromises the safety of spinal anesthesia. Hemorrhagic signs at the site of intramuscular injection were extremely rare.

A randomized, multicenter, assessor-blind clinical trial has been conducted by Di Carlo et al [22] to assess the efficacy and safety of DS, as compared with unfractionated heparin, in the prevention of postoperative venous thromboembolism in patients

with cancer. Patients undergoing elective abdominal, thoracic, gynecologic or urologic surgery for cancer resection were recruited at 27 Italian hospital surgical units. Patients were randomized to DS (600 mg intramuscularly on the second day before surgery, then 300 mg once daily), or calcium heparin (5,000 IU subcutaneously t.i.d., starting 2 hours before operation). Both treatments were continued until at least postoperative day 7, or until adequate mobilization was achieved. Bilateral venography was scheduled at the end of treatment.

Median duration of prophylaxis was 10 days with DS and 8 days with heparin, reflecting the earlier preoperative onset of treatment with the former drug.

Efficacy was assessed in 521 patients with adequate venography and/or confirmed pulmonary embolism. Postoperative venous thromboembolism occurred in 15% patients on DS versus 22% patients on heparin ($p=0.033$). Relative risk reduction was 32.7% (95 CI, 3.1 to 53.2%). The number of patients needed to treat with DS, instead of heparin, to prevent one thromboembolic event was 14. There were no between-treatment differences either in bleeding complications nor in intraoperative blood loss, or in the volume of intra- and postoperative transfusions.

Six cases of thrombocytopenia were identified: 2 on DS with no associated clinical symptoms, and 4 on heparin which were associated with acute arterial thrombosis in one patient and with a bleeding episode in another.

During hemodialysis substantial anticoagulation is required to maintain the patency of the circuit, which is usually achieved with heparin.

A series of three consecutive dose-finding studies on DS was conducted by Nurmohamed et al [23] in a total of 25 patients undergoing a 3-4 h dialysis session.

A further series of four dose-finding studies was carried out by Ryan et al [24] in a total of 30 patients undergoing a longer duration of dialysis (5-7h).

The analysis of dialysis duration without (or prior to) heparin administration, as well as the laboratory results consistently indicated that the optimal regimen of DS was 5 mg/kg bolus plus 5 mg/kg as a 5 h infusion.

The chronic use of DS was investigated in 17 patients consecutively dialyzed with DS for at least 6 weeks, twice or three times a week (Nurmohamed et al) [25]. DS was administered as a single pre-dialysis bolus, starting from 5 mg/kg. Once the optimal dose was reached, the efficacy of DS remained constant for the entire duration of the study. Two patients were withdrawn from the study after presenting an anaphylactoid reaction a few minutes after DS bolus injection.

A further study was performed by Boccardo et al [26] to investigate the chronic efficacy and safety of individually adjusted doses of the currently marketed DS on ten patients on maintenance hemodialysis for chronic renal failure. Patients underwent three consecutive investigation phases. In phase 1, the individual dose was identified; in phase 2, individualized DS doses were validated by a randomized crossover comparison with the

individual heparin dose of each patient (range, 65-134 IU/kg per dialysis), in phase 3, each patient underwent 24 consecutive dialyses with DS over 8 weeks. A total of 275 dialysis sessions were investigated. No statistically significant differences were found between DS and heparin and no bleeding, thrombocytopenia or other adverse events were observed. Thus, as with heparin, the doses and the administration regimen may vary according to the thrombogenicity of the dialyzer filter, duration of the procedure and individual patient characteristics.

LMW-DS, given as an i.v. bolus followed by continuous infusion, has been clinically tested in pilot studies on the treatment of VTE (Kretz et al [27]; Venosi et al [28]; von Kemp et al [29]). No adverse effects were observed in the three studies. Since LMW-DS shows a higher bioavailability than the natural compound after s.c. administration (Saivin et al) [30], the efficacy of LMW-DS given s.c. may deserve further study.

The DS-allied compounds sulodexide and mesoglycan have been clinically studied in a number of trials. The studies on sulodexide published before 1998 have been reviewed by Harenberg [31] together with the pharmacological properties of this compound.

Two recent papers by Coccheri et al [32, 33] show evidence of efficacy of 60 mg i.m. and 100 mg oral sulodexide in the treatment of venous ulcers and intermittent claudication. Similarly, mesoglycan has been studied in the past in different arterial and venous diseases and, more recently, for the treatment of PAD (Nenci et al) [34], in combination with aspirin, and of venous ulcers (Arosio et al). [35]

In both studies mesoglycan (or placebo) was given 30 mg i.m. for 3 weeks followed by 100 mg/day orally. Treatment with mesoglycan improved significantly the walking capacity and tended to reduce the rate of ischaemic cardiovascular events ($p=0.053$) in patients with PAD, and resulted in a significantly faster and more frequent ulcer healing in patients with venous ulcers. Treatment was well tolerated, even in conjunction with aspirin.

As with sulodexide, mesoglycan extended its effect far beyond the period of i.m. administration. Moreover, much of the treatment related differences developed during oral therapy and it is unlikely that they represented a carry-over phenomenon from the i.m. period: a contribution of the oral treatment to results, therefore, is likely; indeed, sulphated polysaccharides are fractionally absorbed after oral dosing and can produce "in vivo" antithrombotic effects while generating barely detectable anticoagulant activities (Costantini et al) [36].

Conclusions

In conclusion, effectiveness and safety of DS have been proved for the prevention of VTE and shown that they can favourably compete with those of UFH in the difficult setting of cancer surgery. In patients sensitized to heparin, DS may be used to perform haemodialysis, or to treat HIT if hirudin is not available.

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