Dermatan Sulphate Induces Plasminogen Activator Release in the 
Perfused Rat Hindquarters

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Heparin or heparin-like substances have been described to 
induce the release of plasminogen activator (PA) activity in 
different animal perfusion models. In this paper we report 
that Dermatan Sulphate (DS) is able to induce PA activity 
release in the perfused rat hindquarters. Perfusion of 
different doses of DS (0.1 to 0.8 mg/mL) stimulates a 
release of PA activity that is maximum after the initial two 
minutes of perfusion. The amount of PA activity released 
rises progressively within a certain concentration range of 

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MATERIALS AND METHODS

Dermatan Sulphate (DS) MF 701, isolated from pig intestinal 
mucosa, was kindly supplied by Mediolanum Farmaceutici SRL, 
Milano, Italy. Heparin contamination was <1% as demonstrated by 
electrophoretic analysis and the in vitro anticoagulant activity was 
calculated to be around 5 U/mg USP.

Perfusion Model

The experimental model used was the perfused rat hindquarters 
model of venous thrombosis,8,9 without affecting the coagulation tests. To clarify the possible mechanisms that may 
contribute to the antithrombotic efficacy of DS, we studied 
its interaction with the drug's antithrombotic system. In 
particular, we investigated if DS was able to modify the 
release of plasminogen activator (PA) from the vascular bed 
using the perfused rat hindquarters model. This model was 
proposed8 as a useful tool to study the physiological and 
pharmacological modulation of PA release.

Experimental procedure. Each experiment started with a 30 
minute, wash-out period of the rat hindquarters using only Tyrode’s 
solution. After this initial period, the drug under investigation 
dissolved in Tyrode’s) was perfused for three minutes, followed by a 
second wash-out with Tyrode’s solution. Samples (1 mL) were 
collected at regular intervals (five to ten minutes) from the cannu-
ulated vena cava during wash-out and every minute during drug 
perfusion. The samples, collected on ice, were centrifuged (12,000 g 
for 60 seconds) and analyzed for fibrinolytic activity. Each animal 
was perfused only once.

Fibrinolytic Activity

Fibrin plate assay. PA activity of the samples, collected during 
the perfusion period, was measured by a fibrin plate method using 
plasminogen-rich human fibrinogen (Grade L; Kabi, Stockholm).11

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Thirty microliters of each sample were placed (in duplicate) on the fibrin film and the diameter of the lysis areas were measured after 18 hours incubation at 37°C. The PA concentration was expressed as PA mU/mL of perfusate in comparison to a reference curve obtained, for each set of experiments, using known concentrations of t-PA standard (WHO, London, coded 831517). The results were analyzed statistically by Duncan’s test.12

Fibrin autography. The perfusion samples, collected as described in the experimental procedure, were kept at -20°C in 0.01% Tween 80 until tested. The molecular type of PA was evaluated by a fibrin autography technique13 after SDS-PAGE. The apparent mol wt of PA was calculated by comparing the migration distance of the lysis area with those of marker proteins of known mol wt (Pharmacia, Uppsala, Sweden), run simultaneously. The immunological characterization was performed with home-made rabbit antibodies against human t-PA or UK incorporated in the fibrin agar plate.

RESULTS

Using the rat hindquarters perfusion model, we observed that DS was able to stimulate the release of PA activity in a dose dependent manner (in the range of 0.1 to 0.4 mg/mL). At higher DS concentrations (0.6 to 0.8 mg/mL) there followed a gradual decrease in the lysis of fibrin plates (Fig 1). Throughout the whole of these experiments no increase in basal perfusion pressure (50 to 60 mmHg) was recorded.

If we examine the profile of PA activity measured in the perfusate with the DS dose giving the highest PA activity release (0.4 mg/mL), we can observe (Table 1) that the maximum PA level was achieved during the first initial minutes of perfusion progressively declining thereafter to basal values 50 to 60 minutes from the beginning of the experiment.

Table 1 reports PA activity released from vascular cells during the perfusion of DS at doses of 0.1 to 0.4 mg/mL. The release of PA activity induced by DS (0.2 to 0.4 mg/mL) was maximal and significantly different from baseline levels during the first two minutes of DS perfusion and decreased thereafter. Above 0.4 mg/mL a reduction in PA was also seen, 0.6 mg/mL inducing a similar release of PA as the 0.1 mg/mL dose.

Table 1. PA Activity Released in Rat Hindquarters Perfused With Different DS Concentrations

<table>
<thead>
<tr>
<th>Perfusion Time (min)</th>
<th>PA Released (mU/mL) After DS Perfusion*</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>0.1 mg/mL</td>
</tr>
<tr>
<td>25</td>
<td>Tyrode’s</td>
</tr>
<tr>
<td>30</td>
<td>DS</td>
</tr>
<tr>
<td>31</td>
<td>DS</td>
</tr>
<tr>
<td>32</td>
<td>DS</td>
</tr>
<tr>
<td>35</td>
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<td>40</td>
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<tr>
<td>50</td>
<td>Tyrode’s</td>
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<tr>
<td>60</td>
<td>Tyrode’s</td>
</tr>
</tbody>
</table>

Note. n, numbers of animals; mg/mL, dose of DS.

*Mean ± SEM.
†P < .01 Duncan’s test (v Tyrode’s sample collected after 25 minutes).

Tyrode’s and DS perfusion were examined by fibrin autography after SDS-PAGE to determine the type of PA released. The perfusate contained two types of PA, which appeared on a fibrin-agar plate as lysis bands of 67,000 and 43,000 mol wt (Fig 2). It can clearly be seen, that both in control samples (collected during the first wash-out period) and in the stimulated samples (collected in the first minute of DS perfusion), both bands appeared and that perfusion of DS caused an increase only in the band corresponding to a 67,000 mol wt while the lower mol wt band was unaltered. The 67,000 mol wt band is blocked by an anti–PA antiserum and the 43,000 mol wt band partially inhibited by an anti-human UK antibody, as previously described.14

To verify whether DS had a direct fibrinolytic activity on fibrin films or an in vitro interaction with PA molecules, DS...
(0.1 to 0.8 mg/mL) was dissolved in Tyrode's solution and placed directly on a fibrin plate, alone or together with different concentrations (10 to 100 mU/mL) of standard t-PA. DS alone did not lyse and did not potentiate the activity of standard t-PA. Moreover, no increased activity was observed when Tyrode's perfusion fluid was used as a source of PA instead of t-PA standard (data not shown).

DISCUSSION

DS is one of the components of the extracellular matrix; it is found alone or in association with other glycosaminoglycans in human coronaries, in bovine and Rhesus monkey aorta. Unlike heparin, DS inhibits thrombin formation by interacting with only heparin cofactor II and not with antithrombin III. Recently, it was shown that DS displays antithrombotic activity in models of experimental venous thrombosis.8,9 In contrast to heparin, the antithrombotic activity was achieved at doses devoid of hemorrhagic effects. These characteristics make DS a potentially interesting molecule for thrombosis prevention and call attention to its role in the mechanism of its peculiar antithrombotic effect. This paper reports the original observation that perfusion of DS induces an increase in fibrinolytic activity in the rat hindquarters model. The increase was dose-dependent, in a range of 0.1 to 0.4 mg/mL, but decreased following a further increase in the doses used. The rise in fibrinolytic activity was concomitant with the presence of DS in the microvasculature; maximum release was achieved during the initial minutes of perfusion followed by a slight decrease during the subsequent minutes (Table 1). The fibrinolytic activity induced by DS has been identified as t-PA-like molecules on the basis of the relative mobility in SDS-PAGE.

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