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REGULAR ARTICLE

Insight into the profibrinolytic activity of dermatan sulfate: Effects on the activation of plasminogen mediated by tissue and urinary plasminogen activators

María Mercedes Castañon*, Cecilia Gamba, Lucía C. Kordich

Laboratorio de Hemostasia y Trombosis, Departamento de Química Biológica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Buenos Aires, Argentina

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Abstract

Introduction: Dermatan sulfate (DS) is well-known for its anticoagulant activity through binding to heparin cofactor II to enhance antithrombin action. It has also been suggested that DS has a profibrinolytic effect, although the exact molecular mechanism is as yet unknown.
Materials and methods: An in vitro amidolytic method was used to study the effect of high and low molecular weight-DS on the activation of Glu and Lys-plasminogen by tissue and urinary plasminogen activators (t-PA and u-PA).
Results: Both high and low molecular weight-DS exhibited a stimulating effect on the activation of plasminogen by PAs. Interestingly, high molecular weight-DS stimulated Glu and Lys-plasminogen activation by t-PA and u-PA in a way and to an extent similar to that in which fibrin(ogen) degradation products (PDF) increased the t-PA assay. Meanwhile low molecular weight-DS had a lower effect. No DS had any effect on plasmin or u-PA amidolytic activity. The facilitation of the conversion of Glu-plasminogen to plasmin in the presence of DS was confirmed by SDS-PAGE; high molecular weight-DS effect was greater than low molecular weight-DS in accordance with the chromogenic assays. Moreover, the combination of PDF and high and low molecular weight-DS,

Abbreviations: Plm, plasmin; Plg, plasminogen; PA, plasminogen activators; t-PA, tissue plasminogen activator; u-PA, urinary plasminogen activator; DS, dermatan sulfate; GAG, glycosaminoglycan; MW, molecular weight; HC II, heparin cofactor II; EACA, 6-aminohexanoic acid; HMW-DS, high molecular weight dermatan sulfate; LMW-DS, low molecular weight dermatan sulfate; PDF, home-made human fibrin(ogen) fragments; pNA, p-nitroaniline.

* Corresponding author. Departamento de Química Biológica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Pabellón II, piso 4°, Ciudad Universitaria (C1428EHA), Buenos Aires, Argentina. Tel./fax: +54 11 4576 3342.
E-mail address: mamecas@qb.fcen.uba.ar (M.M. Castañon).

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respectively, did not further stimulate t-PA activation of either Glu or Lys-plasminogen suggesting that both substances may compete for the same binding sites.
Conclusions: Through in vitro assays we demonstrated that high and low molecular weight-DS enhance plasminogen activation by u-PA and t-PA, suggesting that the profibrinolytic activity of DS might be via potentiation of plasminogen conversion to plasmin.

Introduction

Plasmin (Plm), the main enzyme of the fibrinolytic system, is formed from plasminogen (Plg) by action of plasminogen activators (PA), such as tissue plasminogen activator (t-PA) or urinary plasminogen activator (u-PA), also called urokinase. t-PA exerts its effect primarily on the vascular system and is the main agent for the dissolution of thrombi via activation of clot-bound Plg to Plm, whereas u-PA is widely present in connective tissue and plays an important role in cell migration and tissue remodeling. The activation of Plg by t-PA is strongly enhanced in the presence of fibrin, while the activation by u-PA is barely influenced [1]. The native form of Plg (Glu-Plg) has an NH_2-terminal glutamic acid. The action of Plm on Glu-Plg results in the cleavage of the NH_2-terminal preactivation peptide by hydrolysis of one or several of the following bonds: Arg68–Met69, Lys77–Lys78, or Lys78–Val79 and the final product obtained is Lys-Plg. Lys-Plg is more easily activated to Plm than Glu-Plg [2].

Dermatan sulfate (DS) is an endogenous sulfated glycosaminoglycan (GAG) consisting of repeated units of alternating residues of *N*-acetylgalactosamine and uronic acid (mainly L-iduronic acid) [3]. Dermatan sulfate is produced by fibroblasts and is not detected in normal adult plasma, although the presence of DS was described in the plasma of pregnant women at term and their fetuses [4], in patients on chronic hemodialysis [5], in acutely burnt [6] and septic patients [7]. Dermatan sulfate has the capacity of enhancing the inactivation of thrombin and meizothrombin by heparin cofactor II (HC II) [8,9]. Proposed physiologic roles of DS–HC II include extravascular thrombin inhibition, inflammatory signaling, and thrombin regulation during pregnancy [10]. As DS is a heteropolysaccharide, the molecular weight (MW) is better expressed as its mean number. Commercially available DSs are high and low molecular weighted, 20 to 40 kDa and 3 to 6 kDa, respectively, being the former the native isoform [11]. Dermatan sulfate has been shown to be clinically effective and safe in the prophylaxis and treatment of venous thromboembolism [12–16], with a lower incidence of bleeding complications than heparin. It has also been successfully administrated as an anticoagulant to patients with heparin-induced thrombocytopenia [17,18] and in hemodialysis for chronic renal failure [19,20]. Studies in animal models evidence that DS not only inhibits thrombus formation or growth [21–24], but also causes a marked weight reduction of preformed thrombi in a time and dose dependent way [25–27]. Interestingly, it was reported that the reduction of thrombus weight was independent of the inhibition of thrombus accretion [28] and that it was significantly attenuated by pretreatment with the fibrinolytic inhibitors, 6-aminohexanoic acid (EACA) or lipopolysaccharide [25–27]. Prevention of thrombus formation may be related to the inhibition of blood coagulation, whereas the induction of thrombus reduction is mainly due to an anticoagulant-independent mechanism likely involving the endogenous fibrinolytic system. Since DS was not associated to enhanced systemic fibrinolysis [25–27,29–31], it was postulated to be involved in the local enhancement of the fibrinolytic process. Related to this additional profibrinolytic action of DS, it was reported that DS induces t-PA release from endothelial cells in vivo [32], enhances the lysis of laser-induced thrombus in vivo [33], reduces the level of plasminogen activator inhibitor-1 in cultured human umbilical vein endothelial cells [34] and enhances the activation of Plg by t-PA [35].

Concerning other GAGs in fibrinolysis, it was reported that heparin, heparan sulfate, and dextran sulfate enhanced Plg activation by t-PA and u-PA [36,37]. Heparin binds to various components of the fibrinolytic system, with tight binding to t-PA, u-PA and Lys-Plg and weaker binding to Glu-Plg [38]. Heparin binding sites were mapped to similar fibrin regions on the t-PA molecule [39] (finger and kringle-2 domain) and on the kringle domain of u-PA [40]. The hypothesis that heparin might act as a surface that brings Plg and PA together, facilitating and accelerating the conversion to Plm was proposed as the molecular mechanism of action [36,38]. However, Liang et al. [41] showed that the stimulation of t-PA activity did not seem to follow a template model, and proposed that probably the heparin effect is due to a direct binding to t-PA, causing a conformational change and rendering it more accessible to Plg interaction.