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The influence of various structural parameters of semisynthetic sulfated polysaccharides on the P-selectin inhibitory capacity

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Abbreviations:
BCECF-AM, 2′,7′-bis(2-carboxyethyl)-5,6-carboxyfluorescein-acetoxymethyl ester
CurS, curdlan sulfate
DP, degree of polymerisation
DS, degree of sulfation
ESI-MS, electrospray ionisation mass spectrometry
LMWH, low molecular weight heparin
mAb, monoclonal antibody

ABSTRACT

Selectin-mediated leukocyte rolling along the endothelium is of key importance for maintaining the cellular immune response. The anti-inflammatory activities of heparin have partly been related to inhibition of P-selectin binding. Heparin, however, suffers from its heterogeneous variable structure, the animal origin and multiple *in vivo* effects. As P-selectin is a promising target for anti-inflammatory approaches, we focused on P-selectin inhibition by other sulfated polysaccharides and compared them with six heparins. We examined 15 structurally defined semisynthetic sulfated glucans, non-animal-derived from the linear glucans phycarin, curdlan or pullulan. The derivatives gradually differ in their degree of sulfation, molecular weight, and glycosidic linkage. The inhibitory capacity was analysed in a parallel plate flow chamber, detecting the rolling of U937 cells on P-selectin layers.

Unfractionated heparins displayed variabilities between different preparations. Considering fractionated heparins, exceeding of a minimal mass is essential for activity. Comparing the glucan sulfates, charge density is the most important parameter for P-selectin binding. Highly sulfated derivatives are excellent inhibitors, the reduced cell binding up to $16.2 \pm 6.4\%$ strongly exceeded the heparin activities. Molecular weight is of minor effects, while glycosidic backbone linkage holds certain importance.

To check the P-selectin inhibition *in vivo*, heparin and one phycarin sulfate were tested using intravital microscopy of microvasculature in mice. Both compounds significantly reduced the rolling fractions of activated platelets on endothelium as effective as a blocking P-selectin antibody.

Our study indicates that semisynthetic glucan sulfates with optimal structures block P-selectin excellently and might become promising candidates for anti-inflammatory drugs to replace heparin for certain applications.

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MMWH, medium molecular weight heparin
MW, molecular weight
PhyS, phycarin sulfate
PGE_1, prostaglandin E_1
PSGL-1, P-selectin glycoprotein ligand-1
PulS, pullulan sulfate
RT, room temperature
sLex, sialyl $Lewis^X$
TRAP, thrombin receptor agonist peptide
U937, human monocytic cell line
UFH, unfractionated heparin
VLMWH, very low molecular weight heparin

1. Introduction

The recruitment of leukocytes from the blood stream into tissues is a highly orchestrated process, which proceeds in postcapillary venules of most organs in a cascade-like fashion [1]. It is initiated by capturing of flowing leukocytes (tethering), which then roll along the endothelial surface as a functional prerequisite for the subsequent firm adhesion and transmigration. Tethering and rolling is mediated by selectins, a family of three carbohydrate-binding receptors on both endothelium and leukocytes [2]. They are transmembrane glycoproteins which share a highly conserved N-terminal lectin domain [3]. According to their different kinetics of expression, the three selectins have various, although overlapping functions in the inflammatory reaction. P-selectin is rapidly mobilised to the surface of endothelial cells and platelets upon a variety of stimuli and is thus important for the early phase of leukocyte extravasation [4].

However, dysregulation, i.e. responding to autogenous or non-threatening factors, may lead to an uncontrolled excessive infiltration of leukocytes into healthy tissue. Numerous model experiments using selectin-deficient animals have clearly proven that selectins are implicated in the development of pathological inflammations such as rheumatoid arthritis, asthma, inflammatory bowel disease and psoriasis [5,6]. Furthermore, selectins are important in atherosclerosis and cancer metastasis [7,8]. Consequently, the blocking of selectins has attracted much attention during the last decade as a promising strategy for therapeutic interventions.

Since the minimal binding structure recognised by all selectins, the tetrasaccharide sialyl $Lewis^X$ (sLex) [9] showed to display certain anti-inflammatory activity [10,11], sLex was regarded as lead structure for extensive drug research [12]. However, the very low binding affinities of sLex (K_d 0.1–5.0 mM) [13,14] could only be slightly improved by structural modifications. Presently, only few compounds are investigated in advanced preclinical or clinical trials [15].

Insight into the molecular binding characteristics was gained by analysing the crystal structure of P-selectin complexed with its natural ligand PSGL-1 [16]. In addition to a certain carbohydrate pattern, sulfated tyrosines revealed to dominantly contribute to the binding. The importance of electrostatic interactions for P- and L-selectin binding is in line with former results by Skinner et al. [17,18], showing that sulfated polysaccharides such as fucoidan, dextran sulfate or heparin bind to P-selectin.

Heparin belongs to the group of vertebrate glycosaminoglycans and is a complex, highly sulfated polysaccharide mixture with a molecular weight (MW) between 3000 and 30,000. Heparin has been used as antithrombotic drug for more than 65 years, but it also displays many other biological activities, amongst others anti-inflammatory effects [19,20]. The anti-inflammatory efficacy of heparin-derivatives are currently investigated in clinical trials [21]. Both the anti-inflammatory and anti-metastatic effects of heparin are assumed to be at least partly due to its P-selectin blocking capacity [7,22]. Initial studies indicated that heparins can act as ligands for P- and L-selectin and thus interfere with sLex-related structures [17,18,23,24].

A detailed understanding of the molecular mechanisms of heparin–selectin interactions is an important prerequisite for therapeutic approaches. As heparin exhibits strong antithrombin-mediated anticoagulant activities, the risk of bleeding may limit its therapeutic use in inflammation or cancer treatment. A major challenge is therefore to find heparin-like structures binding to selectins, but exhibiting less anticoagulant activity. Meanwhile, several studies show that the antithrombin- and selectin-binding properties of heparins can be controlled by structural modifications [24]. Xie et al. and Gao et al. used carboxy-reduced heparins and demonstrated a loss in anticoagulant activity while retaining P-selectin binding due to sulfation [25,26]. Wang et al. and Wei et al. focused on the influence of the sulfation pattern of heparin on its P-selectin binding. They proved 6-*O* sulfation of heparin to be essential, while 2,3-*O* desulfation retains selectin binding activity but strongly reduce anticoagulation [27,28]. But the fact that the natural heparin represents a highly variable complex mixture might complicate the specification of structure activity relationships.

The heparin findings focus a strong interest on other sulfated polysaccharides as potential selectin inhibitors. We recently compared the influence of heparin on selectin-