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Article type : Review Article

Using heparin molecules to manage COVID-2019

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This work is supported in part by NIH grants (HL094463, HL144970, HL146226 and HL142604)

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the [Version of Record](#). Please cite this article as [doi: 10.1002/rth2.12353](https://doi.org/10.1002/rth2.12353)

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Abstract

The COVID-19 (coronavirus disease 2019) pandemic is becoming one of the largest global public health crises in modern history. The race for an effective drug to prevent or treat the infection is the highest priority among healthcare providers, government officials and the pharmaceutical industry. Recent evidence reports that the use of low-molecular weight heparin reduces mortality in severe coronavirus patients with coagulopathy. Although the full scope of the benefits from heparin for COVID-19 patients is unfolding, encouraging clinical data suggest that heparin-like molecules may represent a useful approach to treat or prevent SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) infection. This article intends to offer our opinions on the mechanism(s) by which heparin may attenuate the course of SARS-CoV-2 infection. Furthermore, we propose a novel strategy to treat or prevent SARS-CoV-2 infection using 'designer' heparin molecules that are fabricated using a synthetic biology approach.

Essentials:

- Administration of low-molecular weight heparin is beneficial to severe COVID-19 patients, but the mechanism is unknown.
- Heparan sulfate may bind to SARS-CoV-2 spike protein to block viral attachment or entry.
- Heparan sulfate attenuate inflammation responses through neutralizing the activity of proinflammatory proteins, i.e. histone and HMGB1.
- Use of specially designed heparan sulfate oligosaccharides offer a new strategy to manage COVID-19.

The recent COVID-19 (Coronavirus Disease 2019) pandemic caused by SARS-CoV-2 (Severe Acute Respiratory Syndrome Coronavirus 2) infection has been associated with enormous economic and social disruption around the world. There is an urgent need to find therapeutic solutions to curb the spread of the infection and effectively treat established disease. Heparin is a widely used anticoagulant. It interacts with many proteins due its molecular structure and as such, may play a role in the effort to combat SARS-CoV-2 infection. Recent reports have shown that hospitalized patients with severe COVID-19 complicated by coagulopathy experienced reduced mortality when receiving low-molecular weight heparin (LMWH) [1]. This has led to the recommendation to use LMWH for the management of coagulopathy in COVID-19 by the International Society on Thrombosis and Haemostasis (ISTH) [2].

Heparan sulfate (HS) is a sulfated polysaccharide that is present on cell surfaces and in extracellular matrix in the form of HS proteoglycans [3] (Fig 1). HS proteoglycan contains a core protein and polysaccharide side chain. HS proteoglycan plays essential roles in maintaining and regulating a wide range of functions, including coagulation activity [4], inflammatory responses [5] and viral entry into target cells [6] (Fig 1). HS polysaccharides consist of a disaccharide repeating unit of glucosamine and iduronic acid or glucuronic acid (Fig 1), and each individual sugar residue carries sulfo groups. While both core protein and HS polysaccharide chains are essential for the functions of HS proteoglycans, the HS chains interact with a wide range of proteins through the negatively charged sulfo groups, while the core protein is the anchor on the cell surface. HS proteoglycans are also present in the extracellular space. In HS polysaccharides, a cluster of saccharide residues, from pentasaccharide to octadecasaccharide (18-mer), are generally required to bind to proteins. Furthermore, the location and number of sulfo groups in the various domains determine the binding affinity to specific proteins.

Heparin and LMWH are widely used anticoagulants to prevent and treat thrombotic disorders. Heparin mediates its anticoagulant activity by interacting with antithrombin thereby inhibiting the activity of thrombin and factor Xa. Pharmaceutical heparin has the same disaccharide repeating unit as HS, and low molecular weight heparin is a depolymerized heparin with shorter saccharide chains. Heparin is a special form of HS, having higher anticoagulant activity, a higher degree of sulfation and more iduronic acid (IdoA) residues than HS.

HS binds to viral surface proteins to allow infection to become established

HS has been implicated as a cellular receptor for several viruses [6]. For example, cell surface HS serves as a binding site for herpes simplex virus to attach to the host cell and establish infection [7].

Blocking the binding to cell surface HS using a soluble form of heparin successfully inhibited herpes simplex virus infection in a cell-based assay [8]. In this case, heparin was believed to act as a decoy receptor, diverting herpes simplex virus to bind to the heparin rather than cell surface HS on host cells. Of note, an anti-infection effect of heparin has been reported for severe acute respiratory syndrome (SARS) coronavirus [9]. One study confirmed that HS proteoglycan binds to coronavirus spike protein, which normally mediates binding to the ACE2 (angiotensin-converting enzyme 2) receptor [10]. Furthermore, Mycroft-West and colleagues have recently reported that COVID-19 spike protein S1 receptor binding domain binds to heparin and induces conformational changes in the spike protein [11]. Thus, existing evidence suggests that heparin treatment might reduce the binding of viral spike protein to cell surface HS proteoglycan, thereby inhibiting initial infection or spread from infected to non-infected cells. In addition, extracellular HS proteoglycans also bind to viral proteins through the HS side chains and potentially serve as a barrier to prevent the viral particle from binding to HS proteoglycans and cell receptors on the host cells [12].

Anti-inflammatory effect of HS

There have been extensive efforts to exploit the anti-inflammatory properties of heparin and heparin derivatives in various disease states [13]. The anti-inflammatory effects of heparin and HS are dependent on multi-faceted mechanisms, including the binding to chemokines and proinflammatory proteins [14]. The two-way inter-connectivity between thrombosis and inflammation is now well recognized [14]. However, whether the anticoagulant and anti-inflammatory activities of heparin can be totally separated is debatable. In a recent study, we demonstrated that a synthetic non-anticoagulant **HS 18-mer** (Fig 2A) protects against acetaminophen-induced acute liver injury in a mouse model [15]. It appears that the **HS 18-mer** neutralizes the proinflammatory activity of high mobility group box 1 (HMGB1) protein, thereby attenuating the amplification of liver injury caused by an overly exuberant host immune response. Interestingly, we found that anticoagulant HS 18-mer did not possess the hepatoprotective effect in the same model, suggesting that anticoagulant activity and anti-inflammatory activity are separable properties. In the ischemia-reperfusion liver injury animal model, another sterile inflammation model mediated by HMGB1[16], we also tested the protective effect of various synthetic HS oligosaccharides. Unexpectedly, we discovered that an anticoagulant **HS 12-mer** (Fig 2) displayed greater hepatoprotection than a non-anticoagulant 12-mer [17]. In sum, the data suggest that both anticoagulant and anti-inflammatory activities of HS are required to protect against liver injury caused by ischemia-reperfusion.

Some severe COVID-19 patients reportedly develop coagulopathy and manifest a disseminated intravascular coagulation (DIC)-like syndrome [1]. Administration of LMWH attenuates end organ damage in a bacterial lipopolysaccharide (LPS)-induced DIC murine model [18]. However, in a randomized clinical trial, unfractionated heparin did not improve survival in patients with sepsis [19].

A novel concept to target pro-inflammatory proteins using non-anticoagulant heparin has been proposed by Wildhagen and colleagues [20]. Administration of LPS induces a systemic inflammatory response, mimicking a critical part of the complex pathology associated with sepsis and DIC [21]. Neutrophils or injured cells release damage-associated molecular patterns (DAMPs), including histones and HMGB1, into the extracellular space and the systemic circulation, triggering the release of an array of pro-inflammatory cytokines [22-24]. Some severely affected COVID-19 patients have manifest a lethal complication known as the 'cytokine storm' [25]. In an uncontrolled retrospective study, Tang and colleagues recently reported that LMWH decreased mortality in COVID-19 patients with coagulopathy compared to those who did not receive any heparin. Although the molecular mechanism for this benefit is not understood, it seems likely that the anti-inflammatory effects of heparin are contributory. Whether the most efficacious ratio of anticoagulant to anti-inflammatory activity of heparin can be further optimized remains to be determined.

Development of a method to synthesize structurally defined HS oligosaccharides

One of the challenges in studying the variety of biologic effects of heparin and HS is limited access to pure HS oligosaccharides with defined sugar chain size and clearly defined sulfo group positions. These structural elements play critical roles in targeting specific proteins to define their biologic and/or therapeutic properties. Unfractionated heparin or HS isolated from biological sources are mixtures of polysaccharides with different sizes and sulfation patterns. LMWH is a depolymerized product of unfractionated heparin. Although both unfractionated heparin and LMWH share the same disaccharide repeating units, LMWH has shorter sugar chains and lower anti-thrombin (FIIa) activity (Table 1). The structural complexity of existing forms of unfractionated heparin and LMWH complicates mechanistic studies. Traditional chemical synthesis of HS oligosaccharides is notoriously difficult, as the technique requires sophisticated carbohydrate chemistry. We developed a chemoenzymatic method to synthesize HS oligosaccharides using a series of enzymes to mimic the biosynthesis of HS within cells. This process has dramatically shortened the time for synthesis and improved the synthetic yield. In one example, a heptasaccharide synthesis was completed in 9 synthetic steps using the chemoenzymatic synthetic approach with an overall yield of 43%. By

comparison, a similar synthesis using a purely chemical approach requires 50 synthetic steps with an overall yield <0.1% [26, 27]. The chemoenzymatic synthesis method is now able to produce an unprecedented large oligosaccharide library, providing a potentially unique opportunity to investigate the use of HS to treat severe inflammatory disorders including perhaps, SARS-CoV-2 infection [28].

One critical advantage of the chemoenzymatic synthetic approach is to the ability to perform structural optimization for a specific biological function. A set of similar but subtly different oligosaccharides can be synthesized and then subjected to the relevant biological tests for efficacy. One successful example was accomplished by our research team to prepare a fully synthetic version of heparin as a substitute for animal-source heparin to improve the safety and reliability of heparin supply chains in US and around the globe [27, 29]. The synthetic approach was next used to synthesize different LMWH constructs, *i.e.* **HS 6-mer** and **HS 12-mer** [30] (**Fig 2A**). The **HS 6-mer** is relatively inexpensive to synthesize with a production cost that is comparable to LMWH. The **HS 12-mer** displays improved pharmacologic properties, *i.e.* by demonstrating increased liver metabolism, which is a distinct advantage for renally-impaired patients, and the anti-FXa activity is completely neutralized with protamine [30].

We have also developed an enzyme-based method to synthesize homogeneous chondroitin sulfate E (CS-E) oligosaccharides [31, 32]. CS-E is a naturally occurring polysaccharide, but the chemical structure of CS-E is distinct from HS (Fig 2B). First, CS-E contains a disaccharide repeating unit of GlcA and 4,6-disulfated *N*-acetyl galactosamine. Second, the glycosidic linkages in CS-E are β 1-3 and β 1-4 linkages, whereas in HS the linkages are β 1-4 (or α 1-4) and α 1-4 (Fig 2). Third, the amino group of the galactosamine residue in CS-E is acetylated, whereas 40% of the glucosamine residues in HS are *N*-sulfated. The availability of CS-E oligosaccharides offers a new dimension to investigate sulfated carbohydrates in biological processes that may also be relevant to COVID-19 and other thrombo-inflammatory disorders. For example, we recently discovered that a CS-E nonadecasaccharide (**CS-E 19-mer**) neutralizes the cytotoxicity of histones and attenuates organ damage caused by systemic inflammation [32]. One potential advantage of using **CS-E 19-mer** is that the oligosaccharide does not cross-react with the pathologic antibody in HIT patients, suggesting it has low risk for heparin-induced thrombocytopenia.

Conclusions

Here, we summarize our opinions on the possibility of using HS or CS-E to treat or prevent SARS-CoV-2 infection. In our view, HS offers two distinct benefits in addition to the anticoagulation.

First, HS interacts with the spike protein on COVID-19 to prevent viral binding to host cellular receptors. Second, HS interacts with chemokines and DAMPs released during infection, thereby inhibiting the proinflammatory activities of these proteins. The recent clinical data from COVID-19 patients with coagulopathy have attributed the beneficial effects of LMWH to its anti-inflammatory effects [1, 2]. Indeed, there is now ample evidence to suggest this may certainly be the case [33]. Although the lack of experimental data is a limit for the present report, the investigation of the mechanism of action for HS and COVID-19 is underway. We believe that the beneficial effects of unfractionated heparin and LMWH can be further enhanced in HS and CS-E compounds with optimized oligosaccharide structure, leading to increased anti-inflammatory and anti-viral properties compared to native, animal-derived heparins.

Competing Interests

JL is a founder and chief scientific officer for Glycan Therapeutics (www.glycantherapeutics.com). Other authors declare no competing interests.

Figure Legend

Fig 1. Function of HS and HS proteoglycan. HS proteoglycan (HSPG) consists of a core protein and HS polysaccharide chains. The core protein contains a transmembrane domain that presents HS on the cell surface. Some HSPGs are present in the extracellular matrix. HS chains interact with antithrombin to interact with Factor Xa (FXa) or Factor IIa (FIIa) to regulate coagulation. These chains also bind to chemokines that recruit leukocytes to participate in inflammation. Many viruses bind to HS on host cell surfaces as the initial step to establishing infection. Chemical structures of the disaccharide repeating unit of HS are shown.

Fig 2. Chemical structures of HS oligosaccharides and CS-E oligosaccharides. *Panel A* shows the structure of HS 6-mer, HS 12-mer and HS 18-mer. HS 6-mer and 12-mer have anti-FXa activity. HS 18-mer does not have anti-FXa activity, but it binds to HMGB1 to display anti-inflammatory activity to protect liver injury induced by acetaminophen. *Panel B* shows the chemical structures of CS-E 7-mer and CS-E 19-mer. The CS-E 19-mer does not have anti-FXa or anti-IIa activities, but it attenuates the organ damage induced by bacteria lipopolysaccharide. The glycosidic linkages in HS and CS-E oligosaccharides are also indicated.

Author contributions

JL, RP and NSK designed the project. Jine Li and KA provided conclusions for unpublished results. JL and NSK wrote the manuscript. All author participated in writing the manuscript and agree with the contents.

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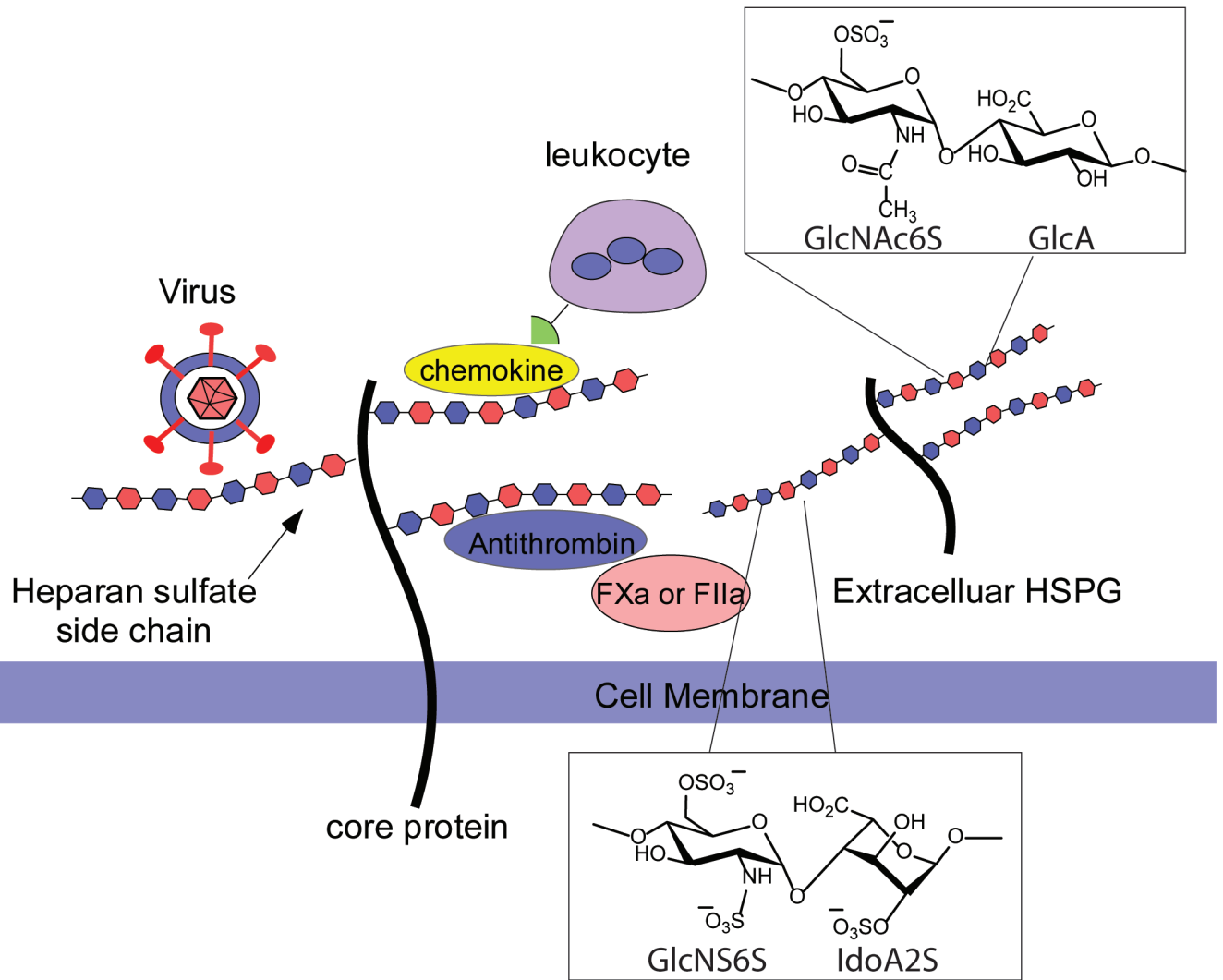
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Table 1. Comparison of unfractionated heparin and different LMWHs*

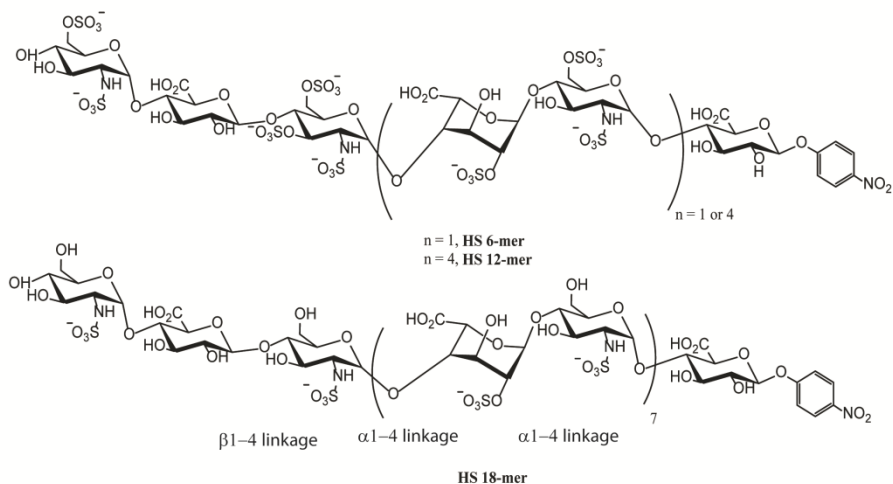
Name	Average MW (Da)	Average size (saccharide units)	Anti-FXa activity (IU/mg)	Ratio anti-FXa and anti-FIIa
Unfractionated heparin	16,000	54	≥180	1.0
Tinzaparin	6,500	22	70-120	1.5-2.5
Dalteparin	6,000	20	110-210	1.9-3.2
Enoxaparin	4,500	15	90-125	3.3-5.3

*Different depolymerization procedures give rise different LMWHs.

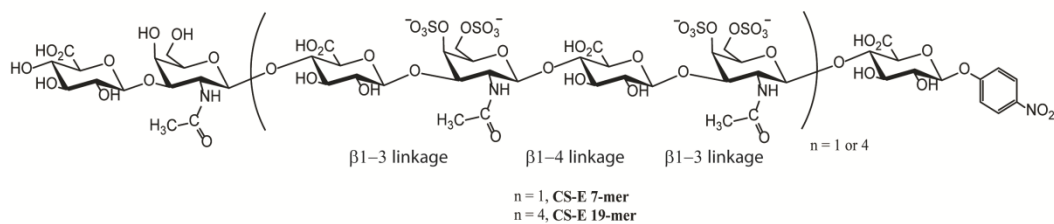


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A. HS oligosaccharides



B. Chondroitin sulfate E oligosaccharides



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