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Using heparin molecules to manage COVID-2019

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Abstract

The coronavirus disease 2019 (COVID-19) 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 health care providers, government officials, and the pharmaceutical industry. Recent evidence reports that the use of low-molecular-weight heparin reduces mortality in patients with severe coronavirus 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 severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection. The intent of this article is 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.

KEYWORDS

chondroitin sulfate, coagulation, COVID-19, heparan sulfate, heparin

Essentials

- Administration of low-molecular-weight heparin is beneficial to patients with severe coronavirus disease 2019 (COVID-19), but the mechanism is unknown.
- Heparan sulfate may bind to severe acute respiratory syndrome coronavirus 2 spike protein to block viral attachment or entry.
- Heparan sulfate attenuates inflammation responses through neutralizing the activity of proinflammatory proteins, that is, histone and high-mobility group box 1.
- Use of specially designed heparan sulfate oligosaccharides offer a new strategy to manage COVID-19.

1 | INTRODUCTION

The recent coronavirus disease 2019 (COVID-19) pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-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 to 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 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] (Figure 1). HS proteoglycan contains a core protein and polysaccharide side chain. HS proteoglycan plays an essential role in maintaining and regulating a wide range of functions, including coagulation activity,[4] inflammatory responses,[5] and viral entry into target cells[6] (Figure 1). HS polysaccharides consist of a disaccharide repeating unit of glucosamine and iduronic acid or glucuronic acid (Figure 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 LMWH 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 residues than HS.

2 | 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 SARS-CoV.[9] One study confirmed that HS proteoglycan binds to coronavirus spike protein, which normally mediates binding to the angiotensin-converting enzyme 2 receptor[10]. Furthermore, Mycroft-West and colleagues[11] recently reported that COVID-19 spike protein S1 receptor–binding domain binds to heparin and induces

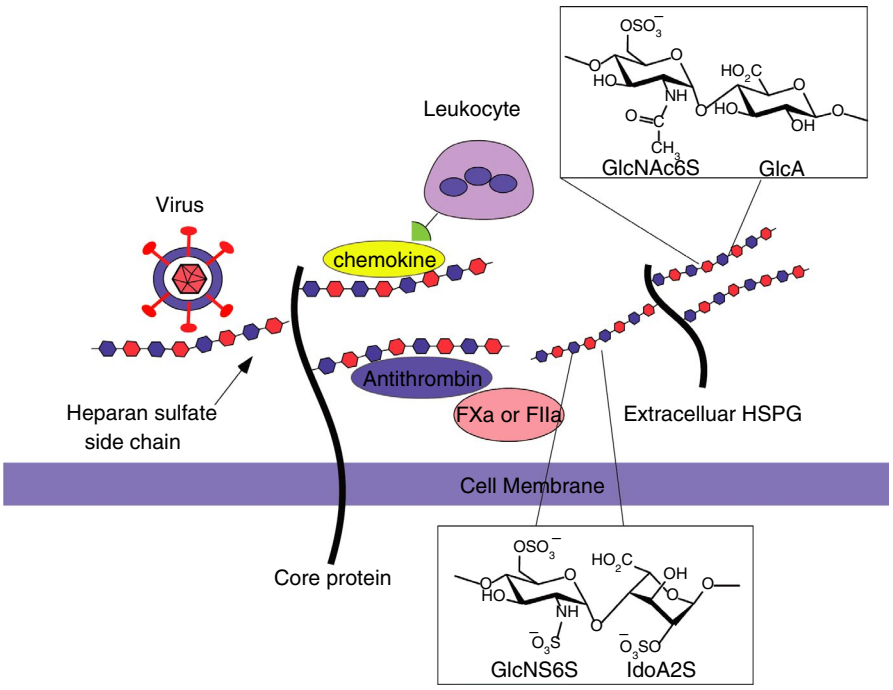

FIGURE 1 Function of heparan sulfate (HS) and HS proteoglycan. HS proteoglycan (HSPG) consists of a core protein and HS polysaccharide chains. The core protein contains a transmembrane domain that presents HS on the cell surface. Some HSPGs are present in the extracellular matrix. HS chains interact 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. GlcA, glucuronic acid; GlcNA, N-acetyl glucosamine; GlcNS, N-sulfo glucosamine; IdoA, iduronic acid

conformational changes in the spike protein. 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 noninfected 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]

3 | 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 multifaceted mechanisms, including the binding to chemokines and proinflammatory proteins.[14] The two-way interconnectivity 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 nonanticoagulant HS 18-mer (Figure 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 (Figure 2) displayed greater hepatoprotection than a nonanticoagulant 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 proinflammatory proteins using nonanticoagulant 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 proinflammatory 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[1] recently reported that LMWH decreased mortality in patients with COVID-19 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.

4 | DEVELOPMENT OF A METHOD TO SYNTHESIZE STRUCTURALLY DEFINED HS OLIGOSACCHARIDES

One of the challenges in studying the variety of biological 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 biological 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 antithrombin (factor IIa) 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 the United States and around the globe.[27,29] The synthetic approach was next used to synthesize different LMWH constructs, that is, HS 6-mer and HS 12-mer[30] (Figure 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 by

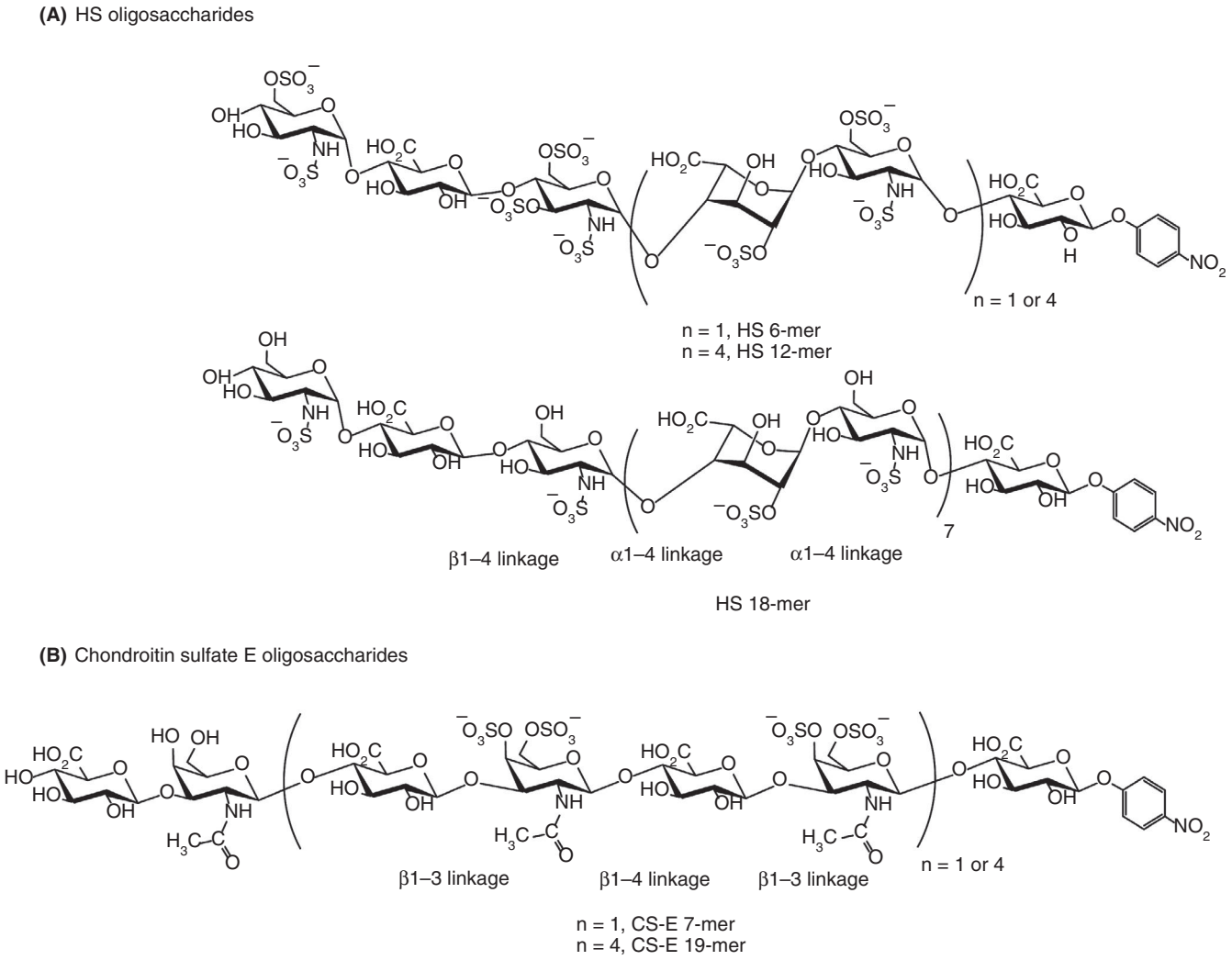

FIGURE 2 Chemical structures of heparan sulfate (HS) oligosaccharides and chondroitin sulfate E (CS-E) oligosaccharides. A, The structure of HS 6-mer, HS 12-mer, and HS 18-mer. HS 6-mer and 12-mer have antifactor Xa (FXa) activity. HS 18-mer does not have anti-FXa activity, but it binds to high-mobility group box 1 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

TABLE 1 Comparison of unfractionated heparin and different LMWHs[a]

Name	Average molecular weight (Da)	Average size (saccharide units)	Anti-FXa activity (IU/mg)	Ratio of anti-FXa and anti-FIIa
Unfractionated heparin	16 000	54	≥180	1.0
Tinzaparin	6500	22	70-120	1.5-2.5
Dalteparin	6000	20	110-210	1.9-3.2
Enoxaparin	4500	15	90-125	3.3-5.3

[a]Different depolymerization procedures give rise different LMWHs.

demonstrating increased liver metabolism, which is a distinct advantage for renally impaired patients, and the anti–factor Xa 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 (Figure 2B). First, CS-E contains a disaccharide repeating unit of glucuronic acid and 4,6-disulfated *N*-acetyl galactosamine. Second, the glycosidic linkages in CS-E are β1-3 and β1-4 linkages, whereas in HS the linkages are β1-4 (or α1-4) and α1-4 (Figure 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 thromboinflammatory 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.

5 | 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 2 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 patients with COVID-19 with coagulopathy have attributed the beneficial effects of LMWH to its anti-inflammatory effects.[1,2] Indeed, there is now ample evidence to suggest that 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 under way. 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 antiviral properties compared to native, animal-derived heparins.

AUTHOR CONTRIBUTIONS

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

RELATIONSHIP DISCLOSURE

J Liu is a founder and chief scientific officer for Glycan Therapeutics (www.glycantherapeutics.com). J Li, KA, RP, and NSK report nothing to disclose.

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