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Review article

Antiviral strategies should focus on stimulating the biosynthesis of heparan sulfates, not their inhibition

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ABSTRACT

Antiviral strategies for viruses that utilize proteoglycan core proteins (syndecans and glypicans) as receptors should focus on heparan sulfate (HS) biosynthesis rather than on inhibition of these sugar chains. Here, we show that heparin and certain xylosides, which exhibit *in vitro* viral entry inhibitory properties against HSV-1, HSV-2, HPV-16, HPV-31, HVB, HVC, HIV-1, HTLV-1, SARS-CoV-2, HCMV, DENV-1, and DENV-2, stimulated HS biosynthesis at the cell surface 2- to 3-fold for heparin and up to 10-fold for such xylosides. This is consistent with the hypothesis from a previous study that for core protein attachment, viruses are glycosylated at HS attachment sites (*i.e.*, serine residues intended to receive the D-xylose molecule for initiating HS chains). Heparanase overexpression, endocytic entry, and syndecan shedding enhancement, all of which are observed during viral infection, lead to glycocalyx deregulation and appear to be direct consequences of this hypothesis. In addition to the appearance of type 2 diabetes and the degradation of HS observed during viral infection, we linked this hypothesis to that proposed in a previous publication.

1. Introduction

Several infectious agents, including viruses, interact with proteoglycans (PGs) on the surface of host cells for attachment [1,2]. Several studies have focused on these interactions, specifically on the surface molecules glycosaminoglycans (GAGs) and heparan sulfate (HS) [1,2]. However, even when viruses interact with HS, the core proteins of most viruses are the viral receptors to which HS covalently attaches (Table 1). This indicates that the observed interaction between HS and certain viruses could be due to their binding to the same elements: the core proteins (Table 1). Many of these viruses use other molecules as coreceptors in addition to the core proteins, as is the case for SARS-CoV-2, which uses syndecans and angiotensin-converting enzyme 2 (ACE 2) as coreceptors [3]. HIV-1 also uses syndecans as well as CD4 as receptors [4]. Hepatitis C virus (HVC) has several other coreceptors in addition to syndecans [5].

The different possibilities for posttranslational modifications (PTMs) of cell surface proteins can explain the ability of viruses to utilize different receptors [25], particularly when considering the diversity of glycoproteins found on viral envelopes and the ability of the viruses to interact with the target host proteins and use these proteins to gain entry into the cell [26,27].

Although most viruses use other receptors on the cell surface in addition to the core proteins, generally, for cell penetration, the core proteins are essential. Bermejo-Jambrina et al. [3] recently showed in an *in vitro* study that SARS-CoV-2 first attaches to heparan sulfate proteoglycans (HSPGs) before interacting with ACE2. However, a recent work by Clausen et al. [28] showed that the spike protein of SARS-CoV-2 could bind simultaneously to the cell surface *via* HS and the ACE2 protein receptor. The consensus of these cited studies is that HSPGs are necessary for attachment of SARS-CoV-2 on the cell surface. Zhang et al. [29] also reached the same conclusion after an *in vitro* study. The essential character of HSPGs for the attachment of SARS-CoV-2 to the cell confirms that HSPGs are a potential therapeutic pathway to be targeted [29].

What was just observed for SARS-CoV-2 by Zhang et al. [29] is also the case for HIV-1. Saphire et al. [4] showed in an *in vitro* study that CD4 alone is insufficient for HIV-1 infection of macrophages and that attachment to HSPGs is also necessary.

This dependence of viruses on HSPGs for cell entry has led to researchers proposing antiviral strategies centered around HS modulation by inhibiting HS expression on the surface of the cell to prevent viral

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Table 1
Viruses, core proteins, and their associations with type 2 diabetes.

Virus	Core proteins of HSPGs	Association with diabetes
SARS-CoV-2: severe acute respiratory syndrome coronavirus 2	Syndecan 1 and 4 as receptors [3,6]	SARS-CoV-2-induced diabetes [7,8]
SARS-CoV	HSPGs are preliminary attachment sites of SARS-CoV [9]	
HCoV-NL63: human coronavirus NL63	HSPGs are critical for HCoV-NL63 binding [10]	
HSV-1: herpes simplex virus type 1	Syndecans 1 and 2 as receptors [11].	Strong association of HSV-1 infection with type 2 diabetes [12]
HPV-16: human papillomavirus type 16	Syndecan 1 as the receptor [13]	Poor prognosis [14]
HVC: hepatitis C virus	Syndecan 1 as the receptor [5]	Strong association between HCV infection and a higher prevalence of type 2 diabetes [15]
HIV-1: human immunodeficiency virus type 1	Syndecans and betaglycans as receptors [4]	HIV is a high risk factor for the prevalence of type 2 diabetes [16]
HCMV: human cytomegalovirus	Hypothesis that syndecans are the receptors of the CMV virus based on an *in vitro* study [17]	Relative risk ratio of up to 12 of having type 2 diabetes for persons previously exposed to CMV [18]
DENV: dengue virus	Syndecan 2 as the receptor [19]	Strong association between diabetes and dengue severity [20]
HTLV-1: human T-cell leukemia virus type-1	HSPGs are critical for HTLV-1 binding [21]	Relative risk ratio of HTLV-1 of more than 5 for persons with type 2 diabetes [22]
HVB: hepatitis B virus HVD: hepatitis D virus	Glypican-5 as the host cell entry factor for hepatitis B and D [23]	HBV is associated with the prevalence of diabetes [24]

attachment or using HS and heparin (Hep) as viral decoys [28–31].

In this article, we explain why HS biosynthesis should be promoted. We also support the hypothesis that the HS attachment sites on core proteins (*i.e.*, the serine (Ser) residues intended to be the receptors of D-xylose for the initiation of HS/chondroitin sulfate (CS) chains) are the probable glycosylation sites of the core proteins where these viruses attach. Indeed, we complement this previously formulated hypothesis by including other supportive facts. In particular, the link to the demonstrated efficacy of Hep and certain xylosides against viruses (listed in Table 1) *in vitro* (see Section 4). The explanation for the overexpression of heparanase (HPSE) observed during viral infections [32–34]. As well as the increased syndecan shedding that leads to the deregulation of endothelial glycocalyx observed during viral infection. Furthermore, we evaluated the link with the endocytic entry of these viruses into cells, particularly the reliance on the O-glycosylation sites on the core proteins (syndecans and glypicans).

2. Context

It is important to note here that in a previous article published in this journal, *Life Sciences*, we showed the importance of stimulating HS biosynthesis to fight inflammation, especially inflammation of the lungs during viral infection. We also formulated the hypothesis mentioned above regarding the attachment site of viruses on core proteins [35]. In Section 5 of the previous paper, this hypothesis allowed us to give a probable explanation of the appearance of type 2 diabetes during viral infection (without mentioning the notion of insulin resistance) and other observed facts. In summary, this hypothesis helped to show how the facts listed below are direct consequences of viral attachment.

(a) The appearance of type 2 diabetes following viral infection, (b) the degradation of sulfated GAGs, primarily HS and CS, observed during both viral infections and type 2 diabetes, (c) the decrease in the activity of xylosyltransferase enzymes, and (d) the increase in hyaluronan.

These explanations were based on several previous aspects and studies, the most important of which are the following: (1) The (unique) position of D-xylose in the linkage region common to the HS, CS and dermatan sulfate (DS) chains; (2) the antiglycemic properties of D-xylose (and xylitol) shown by *in vivo* studies; (3) the properties of D-xylose (as well as D-xylose esters and oligosaccharides containing D-xylose) which stimulate the biosynthesis of sulfated GAGs and PGs, which has been shown *in vitro* and patented by a large French company; (4) Schmidt's work on the importance of countering the degradation of HS during inflammatory processes; and (5) several works on D-xylose/xylitol and viruses/bacteria.

Thus, the association between type 2 diabetes and viral infection listed in Table 1, supporting the abovementioned hypothesis and constituting the basis and link with the previous article. Here, we supplement these facts with additional details listed in Section 1, including feasibility (the fact that HS attachment sites are indeed O-glycosylation sites).

3. Host and viral N- and O-linked glycosylation

There are several PTMs of cell surface proteins, including glycosylation, phosphorylation, and acetylation [36]. Here, we will focus on glycosylation (see Section 4). Glycosylation is an enzymatic reaction that involves the covalent linkage of a carbohydrate to a peptide chain, protein, lipid, or other molecule. The process is the most diverse change (involving covalent bonds) that proteins undergo in the body, both in terms of the types of amino acids that are changed and their structures [37]. Almost all blood serum proteins are glycosylated, and some studies have reported that over 70% of eukaryotic proteins are glycosylated [38,39].

There are several types of glycosylation, with the most common being N-glycosylation and O-glycosylation [40].

N-glycosylation begins in the endoplasmic reticulum (ER) cells through the attachment of a N-bearing oligoside (usually *N*-acetylglucosamine) to an available asparagine (Asn) residue of the target protein that bears the sequence Asn-X-Ser/threonine (Thr), where X represents any amino acid other than proline (P). N-glycosylation occurs in the Golgi apparatus, where tree-structured sugars are fixed to the polypeptide chain, resulting in the formation of glycoproteins. Therefore, it is a cotranslational mechanism [41].

O-linked glycosylation, another PTM, occurs only when the target polypeptide reaches the Golgi apparatus after the biosynthesis of the target polypeptide. O-linked glycosylation begins with the attachment of a sugar molecule to the hydroxyl group of a Ser or Thr residue within the target protein [42]. Some N-linked and O-linked glycosylation sites are on cell surface proteins as potential attachment points that viruses could use for cellular entry.

As with proteins present on the cell surface, glycoproteins found on the viral envelope have several N- and O-linked glycosylation sites. For example, the spike glycoprotein found on the SARS-CoV-2 envelope has several N- and O-linked glycosylation sites. Bagdonaite et al. [43] identified 25 O-glycosites on the ectodomain (ED) of the spike protein, and Shajahan et al. [44] identified 22 potential N-glycosylation sites on the spike protein.

These N- and O-linked glycosylation sites on viral envelopes can be prime targets for vaccine development or therapeutic drugs [45,46].

Potential N- and O-linked protein glycosylation sites are generally predicted by several tools, such as the NetOGlyc 4.0 Server or N-GlyDE (for O- and N-glycosylation sites, respectively) from the target protein sequence [47].

It is important to note that not all Ser and Thr residues are O-glycosylated. Christlet and Velurajo statistically analyzed approximately 1000 protein sequences containing O-glycosylated Ser/Thr residues and showed that potential O-glycosylation sites are mainly dependent on the

proximity of certain amino acids to Ser or Thr residues. For instance, the following regions are potential O-glycosylation sites: (a) a series of adjacent Ser or Thr residues and (b) the presence of a proline residue at the +/−2 positions from a Ser/Thr [48]. However, aromatic amino acids, cysteine, and amino acids with bulky side chains inhibit O-glycosylation [48].

Given the high number of O-glycosites on a single viral glycoprotein, it is understandable that O-linked polysaccharides such as Hep, HS binding peptide, and HS-like proteins bind to various viruses. On the other hand, since all of the viruses listed in Table 1 attach to the core proteins (syndecans and glypicans), we are particularly interested in the O-glycosylation sites on these core proteins, their structures and their shedding.

4. Cell surface core proteins: types, structures, shedding, glycosylation sites, and endocytosis receptors

4.1. Types of cell surface core proteins, their structures, and shedding

There are two types of core proteins to which GAGs are attached to the cell surface (Table 2) The first is transmembrane core proteins, comprising syndecans 1 to 4, betaglycans, phosphacans, CD44, and NG2. Syndecans (1 to 4) are made up of a cytoplasmic domain (CD), a transmembrane domain (TD), and an extracellular ED. The ED is where GAGs (HS/CS/DS/Hep/keratan sulfate (KS)) attach and is also a site of interaction with different viruses. Several enzymes that shed the EDs of syndecans have been identified, including metalloproteinases [49,50].

The second type of core protein consists of membrane core proteins, primarily comprised of glypicans 1 to 6, which are bound to the cell membrane *via* glycosylphosphatidylinositol (GPI). The glypican structure consists of a C-terminal domain close to the GPI link, an HS attachment domain, a globular domain, and an N-terminal signal sequence. Glypicans are released from the cell surface through phospholipase-mediated cleavage of the GPI bond [50,51].

4.2. O-glycosylation sites on core proteins: feasibility of the hypothesis

Section 3 allowed us to recall the definition of O-glycosylation and the characteristics (positions) of O-glycosites. Given our review, the HS attachment sites (*i.e.*, the Ser residues intended to receive D-xylose for the initiation of HS/CS chains on core proteins) are proven sites of O-glycosylation.

Indeed, O-glycosylation initiates HS/CS chains through attachment of the sugar D-xylose to the hydroxyl group of a Ser residue on the core protein by the enzymes xylosyltransferase 1 and 2 (XYLT1, XYLT2) [52]. These HS attachment sites (Ser residues) are also listed as such (*i.e.*, O-glycosylation sites) in the UniProt database (UniProtKB-P18827 (SDC1_HUMAN); UniProtKB-P35052 (GPC1_HUMAN)), which provides protein sequence and functional information, including PTMs [53]. For syndecan 1 (SDC1), the HS attachment sites (Ser residues intended to receive HS/CS) on its ED (residues 23 to 254) are at positions 45 and 47 for HS and 37/207/217 for CS. For glypican 1 (GPC1), these O-glycosylation sites have been established to be at positions 486/488/490 (HS receptors). Since these sites are located closer to the cell membrane for glypicans and because syndecans represent 80% of all core proteins on the cell surface, syndecans are more readily exposed to viruses than glypicans. However, depending on factors such as the O-glycopeptide specificities of different viral glycoproteins and the types and positions of amino acids around O-glycosylation sites (see Section 2), glypicans can still be the predominant receptors of certain viruses (see Table 1).

Table 2
Different core proteins on the cell surface and their release.

Core protein	Type	Main GAG chain	Sheddases or cleavage
Syndecans 1–4	Transmembrane	HS	Shed by metalloproteinases/(HPSE enhances syndecan-1 shedding)
NG2	Transmembrane	CS	Shedding from the cell surface is unknown
CD44	Transmembrane	HS/CS; also hyaluronan (HA) receptors	
Betaglycan	Transmembrane	CS/HS	Shedding from the cell surface is unknown
Phosphacans	Transmembrane	CS	Shedding from the cell surface is unknown
Glypicans 1–6	Membrane	HS	Cleavage of the GPI anchor by phospholipases

HS: heparan sulfate; CS: chondroitin sulfate; HA: hyaluronic acid (hyaluronan).

HS attachment sites, proven O-glycosylation sites, are therefore positions where viral glycosylation is possible. This was indirectly demonstrated by Hudák et al. [6] in an *in vitro* study of SARS-CoV-2. In this study, the researchers investigated the contributions of different parts of one of the cell receptors (syndecan-4) of the spike protein. Studies have shown that the spike protein attaches to two parts of the syndecan 4 ED, with a Mander's overlap coefficient (MOC) of approximately 0.8 for both parts: the portion of the ED of syndecan 4 consisting of HS attachment sites with some HS chains called the HSA and the other portion of the ED, the cell-binding domain (CBD), which does not have a HS chain or HS attachment site [6].

Although the results of the first part of the ED of syndecan-4 corroborate our hypothesis, the results are not enough to affirm it because some HS chains are left in this part of the ED. We may reasonably ask ourselves whether the virus does not instead attach itself only to HS chains and not at all to HS attachment sites, although this has been proven with O-glycosites. The CBD portion does not contain any HS chains or HS attachment sites but does contain only two positions where glycosylation is possible; positions 97 and 101 are proven O-glycosylation sites (a Ser or Thr residue that can receive D-xylose to initiate CS biosynthesis), as shown in the Glygen database [54–56]. This is also true for the 5 identified mutations of syndecan-4, and above all, these positions are the only position in which O-glycosylation is possible in this part of the ED [54] thus supporting our hypothesis, at least for SARS-CoV-2.

4.3. Endocytosis receptors, HPSE, and virus release

The core proteins on the cell surface are internalized by endocytosis after shedding (for transmembrane protein cores) or cleavage of the GPI bond (for glypicans) and degraded by a multistep process ending in the lysosomes [57]. This route of degradation of the core proteins by endocytosis is of particular interest to us since HPSE and heparinase (the enzymes responsible for the degradation of HS) have already been shown to inhibit the attachment to the cell surface of several viruses through several *in vitro* studies, explaining their role in viral entry.

Table 1 lists some viruses that enter the cell through endocytosis.

These viruses include herpes simplex virus (HSV) [58], human papillomavirus (HPV) [59], hepatitis C virus (HVC) [60], human immunodeficiency virus (HIV) [61], human cytomegalovirus (HCMV) [62], dengue virus (DENV) [63], hepatitis B virus [2], and SARS-CoV-2 [64].

Endocytic entry of viruses using the core proteins of PGs as receptors on the cell surface is therefore a consequence of the endocytic degradation of core proteins (see Fig. 1).

In fact, Christianson and Belting showed that HSPGs are cell-surface endocytosis receptors and that the endocytosis of HSPGs is not restricted to a particular pathway but instead varies with context and the extracellular ligand [65]. This could help to better understand the diversity of mechanisms governing viral endocytosis.

The degradation of GAGs *via* endocytosis occurs through HPSE in the endosomes (see Fig. 1a). These enzymes have the ability to dissociate xylose, linking HS/CS to the core proteins, and therefore, these enzymes

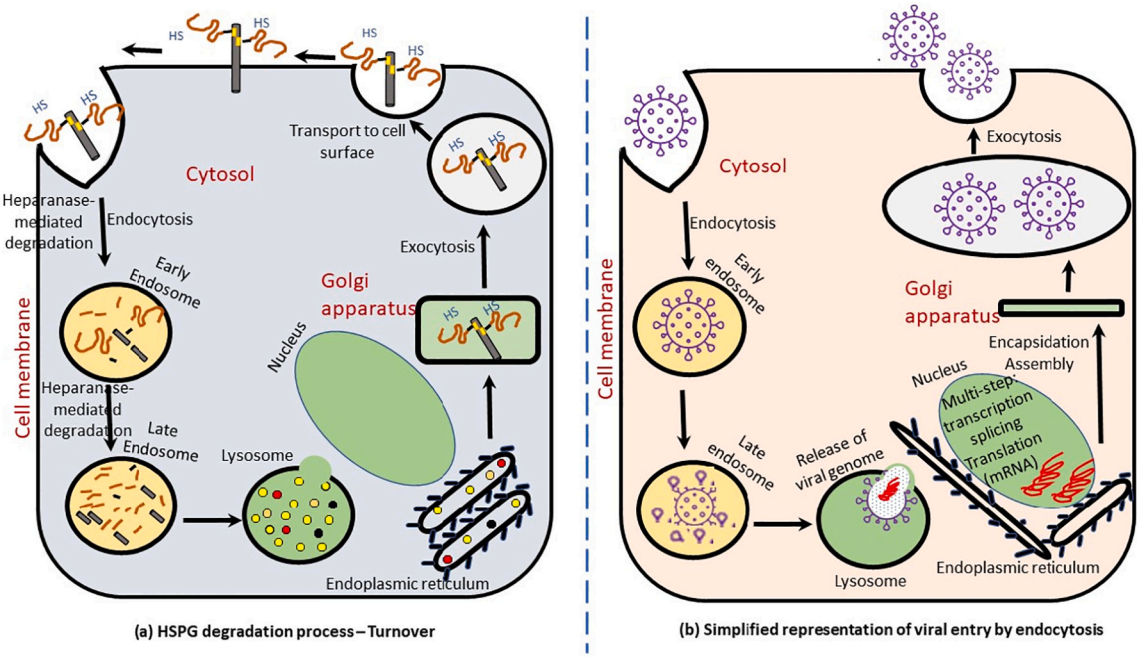

Fig. 1. PG internalization and viral endocytosis. a) HSPG degradation process – turnover. b) Simplified representation of viral entry by endocytosis.

(such as HPSE) probably do the same for a virus attached in the place of xylose (HS/CS attachment site) [65,66].

Several studies have shown that HPSE is an enzyme that is necessary for the release of viruses into the cell. This fact has already been demonstrated for HSV-2 by Hopkins, Yadavalli [67], and the same has been shown for DENV and HPV [33]. In addition, Hadigal and Agelidis [32] demonstrated that HPSE was the host enzyme needed for the release of HSV-1 into the cell. Contrary to the suggestion made by researchers about a potential dual role of HPSE, it seems, as explained above, that HPSE plays a single role: that of the cleavage of HS bonds and therefore of the bond formed by O-glycosylation between HS (or the virus) and the Ser residue on the core protein (HS/CS attachment site).

Viral entry into the cell by endocytosis using core proteins as receptors at the cell surface is a consequence of the internalization of PGs (HS and core proteins) [65]. In addition, the role of HPSE, which is the host enzyme necessary for the release of viruses into the cell, also supports our hypothesis that the HS attachment sites (*i.e.*, the Ser residues intended to receive D-xylose to initiate HS/CS biosynthesis) are the positions where the viruses listed in Table 1 are glycosylated.

4.4. Impact of HS chains on syndecan ED shedding and HPSE overexpression

Infections are usually accompanied by deregulation of the endothelial glycocalyx that surrounds the cell and contributes to its defense. This deregulation of the glycocalyx is usually the result of excessive cleavage of the core proteins present on the cell surface, especially syndecans [68].

Ramani et al. [69] showed in an *in vitro* study that HS chains attached to the core protein SDC1 retain this protein on the cell surface by inhibiting the metalloproteinase enzymes that shed its ED. In addition, the authors demonstrated that HS regulates the biosynthesis of syndecans [69]. These findings indicated that the biosynthesis of HS reduces the shedding of the ED of syndecans, thereby reducing the deregulation of the endothelial glycocalyx and thus corroborating the findings of Schmidt et al. [70]

We have discussed the role of HPSE during viral entry, and several studies have highlighted the overexpression of HPSE in the extracellular matrix (ECM) and on cell surface during the following viral infections: HSV-1, DENV, HPV, and SARS-CoV-2 [33,34]. Some studies have also suggested that HPSE could be taken as a biomarker of the severity of infection for COVID-19 [34].

However, regarding syndecan shedding, Gingis-Velitski et al. [71] showed in an *in vitro* study that HS chains are an important regulator of HPSE. These researchers also noted that cells with less HS absorb less HPSE and thus had more HPSE on the cell surface.

Since the hypothesis identifies HS attachment sites on core proteins as likely sites where viruses attach, stimulation of HS biosynthesis, in addition to preventing viruses from attaching themselves, helps to maintain endothelial glycocalyx by inhibiting the shedding of syndecans. In addition to the inhibition of the metalloproteinases that are responsible for the shedding of the syndecan ED, stimulation of HS chain biosynthesis reduces HPSE on the cell surface. This reciprocally provides an explanation for the mechanism leading to the deregulation of endothelial glycocalyx during viral infection.

5. Antiviral properties of Hep, xylosides, and HS biosynthesis

In a previous article, we described the properties of D-xylose/xylitol, explaining their ability to stimulate the biosynthesis of HS [35]. Therefore, we suggest using the elements listed in Section 2to use them against SARS-CoV-2. Studies have confirmed the antiviral properties of xylitol against SARS-CoV-2.

In fact, Bansal et al. [72] recently demonstrated in an *in vitro* study that xylitol inhibited SARS-CoV-2. In this study, xylitol was used as a placebo but showed great antiviral properties, both alone and in combination with iota-carageen [72]. As mentioned in a previous article [35], these antiviral properties of xylitol against SARS-CoV-2 could be attributed to its ability to stimulate the biosynthesis of HS and the fact that SARS-CoV-2 itself uses syndecans as receptors (see Table 1). Since the Ser residues intended to receive D-xylose for initiation of the biosynthesis of HS are the probable viral attachment points, stimulation of HS biosynthesis will block viral attachment to syndecans, thereby inhibiting cellular entry. This conclusion was also recently formulated by Cannon et al. [73] after an *in vitro* study analyzing the efficacy of a nasal spray containing xylitol against SARS-CoV-2. The researchers showed, through images from the studies, that xylitol inhibited the attachment of this virus to the cell surface.

Another compound known for its multiple medicinal properties against several pathologies and particularly against several viral infections is Hep. There are over 400 Hep-binding proteins that have been reported to date [74]. Most Heps have anticoagulant properties and are often classified under the name low molecular weight heparin (LMWH). One of the commonalities between these different LMWHs is the beginning of their chain (linkage region), which is identical to those of the HS, CS and DS chains, meaning that they all consist of the trisaccharide Xyl-Gal-Gal connected to a Ser or Thr [75,76].

Hep has demonstrated antiviral properties *in vitro* against several viruses, including SARS-CoV-2 and HIV-1 (see Table 3). The diversity of viruses (see Table 3) that have their entry into cells inhibited by this molecule is a strong argument that the effects of Hep could act more on the cell to inhibit the attachment of viruses rather than action on these viruses directly (although Hep binds to most of these viruses - see Section 3). The literature on this molecule in relation to some facts observed during viral infection reports that the addition of Hep or xylosides to cell cultures caused the accumulation of HPSE in the middle of the culture and less accumulation on the surface [71]. Researchers showed during this study that the cellular uptake of HPSE was reduced in HS-deficient cells, inducing an increase in HPSE in the ECM and on the cell surface. Another study confirmed these actions of Hep and xylosides on HPSE at the surface of the cell [77].

Moreover, from our review in the previous sections, we noted the importance of the biosynthesis of HS on the protection of the endothelial glycocalyx and thus on inflammation. We also observed that HS chains regulate HPSE at the cell surface. Properties that are common between Hep and xylosides indicate that Hep could strongly stimulate HS biosynthesis.

In fact, stimulation of HS biosynthesis by Hep reported dates back to the 1980s [95]. Aligned *in vitro* studies on endothelial cells showed that Hep strongly (2- to 3-fold increase) stimulates HS biosynthesis on the cell surface, and this stimulation occurred immediately after endothelial cells were exposed to Hep [95]. Furthermore, Kaji and Sakuragawa [96] concluded that Hep stimulates GAG biosynthesis *in vitro*. A combination of the following facts can explain these observations: (a) the identical linkage regions for HS and Hep, (b) the cleavage sites of HPSE, and (c) the similarities between the HS and Hep chains (both the nature and spatial configuration of disaccharides). In addition, Nader et al. [97] showed that endothelial cell HS contains Hep regions in their structure.

Fransson et al. [98] noticed that the treatment of skin fibroblast cells with Hep led to a reduction in the deposition of PGs in the pericellular matrix and increased the HSPG content on the cell surface *in vitro*. This study, which also included other molecules, led the researchers to conclude that exogenous xylosides enter the ER and are subsequently transported into the trans-Golgi complex for processing and HS production [98]. Therefore, in addition to stimulating the biosynthesis of HS in endothelial cells, Hep stimulates the biosynthesis of HS in fibroblast cells.

In addition, Trindale and colleagues demonstrated through two *in vitro* studies that internalization and degradation of Hep was not required to stimulate HS biosynthesis and that this stimulation could be done from the ECM [99,100]. These findings allowed for generalized Hep-mediated HS biosynthesis stimulation in endothelial cells, fibroblasts, and other types of cells. Furthermore, Gingis-Velitski et al. [71] showed that the addition of Hep or xylose-containing compounds positively impacted HPSE in CHO-745 cells with HS deficiency, corroborating the conclusions drawn from other studies (section 4.4).

6. Discussion

A strong association between type 2 diabetes and viral infections using core proteins (syndecans, glypicans) as receptors has already been demonstrated (see Table 1), as has the degradation (in number) of HS chains during viral infections including HCMV [101], DENV [102], and HSV-1 [32]. The degradation of HS leads to overexpression of HPSE at the cell surface, which is observed during infection (see Section 4.4). In addition, this degradation accelerates the shedding of the ED of syndecans (to which HS is attached) to the cell surface (see Section 4.4), leading to deregulation of the endothelial glycocalyx. The core proteins that are shed or cleaved on the cell surface are internalized into the cell by endocytosis, thus dragging the viral ligand with them. Then, because HPSE is the host enzyme responsible for the release of these viruses into the cell (see Section 4.3) appears to be the consequence of the viruses on PGs combined with the fact that HPSE degrades PGs in the endosome (see Fig. 1).

Since studies have so far shown that these viruses interact with HS (by using core proteins as receptors), several researchers, following *in vitro* studies, have logically suggested therapeutic avenues based on HS inhibition, for instance, the inhibition of SARS-CoV-2 [29].

Table 3
Different viruses inhibited by Hep or other O-linked polysaccharides.

Virus	Antiviral/entry inhibitors	Type of study	Results	Reference
Human respiratory syncytial virus (hRSV)	Xylitol in a dietetic	*In vivo*	Reduction in viral titers	[78]
	Hep-like structures	*In vitro*	Hep-like structures reduces the binding of hRSV to cells (HEp-2 cells)	[79]
SARS-CoV-2	Xylitol	*In vitro*	Virucidal (antiviral properties) (Vero E6 cells)	[72,73]
	Hep	*In vitro*	Hep inhibits cellular invasion by SARS-CoV-2 (Vero cells/ HEK293T cells)	[8,80,81]
HIV-1 (human immunodeficiency virus type 1)	Hep	*In vitro*	Inhibition of HIV-1 replication in cultured cells (T-cell lymphoma line, lymphoblasts)	[82]
	Polysaccharide extract of *Arthrospira platensis*	*In vitro*	Inhibition of HIV-1 replication in human T cell	[83,84]
Hepatitis B virus (HBV)	Hep at high concentrations	*In vitro*	Inhibition of HBV infection (HepG2 cells)	[85]
Hepatitis C virus (HCV)	Hep and highly sulfated HS compounds	*In vitro*	Blocks HVC binding to the cell surface (HepG2 cells)	[86]
Human cytomegalovirus (HCMV) (human herpes virus 5)	HS binding peptide	*In vitro* and *in vivo*	Significantly reduces HCMV infectivity *in vitro* (fibroblast (MRC-5) cells)	[87]
Herpes simplex virus (HSV-1 and HSV-2)	Hep	*In vitro*	Inhibits HSV (leukocyte cells)	[88]
	Polysaccharide extract of Porphyridium spp	*In vitro* and *in vivo*	"Exhibited impressive antiviral activity against herpes simplex virus types 1 and 2"	[89]
Human T-cell leukemia virus type-1 (HTLV-1)	Hep	*In vivo*	Positive effect on patients with HTLV-I-associated myelopathy	[90]
Human papillomavirus (HPV16, HPV31)	Hep	*In vitro*	Inhibition by a highly sulfated form of Hep (keratinocyte cells)	[91]
Dengue virus (DENV1, DENV2)	Hep	*In vitro*	Inhibits viral entry (Vero cells, BHK cells)	[92,93]
	CS E	*In vitro*	Inhibits viral entry (Vero cells, BHK cells)	
Influenza H5N1 (flu)	Hep	*In vitro*	Inhibition of viral invasion	[94]

The multiple negative consequences of the degradation of HS have led us to instead consider the promotion of HS biosynthesis. Because LMWH shows inhibitory properties against diverse viruses, such as SARS-CoV-2, HIV-1, HPV-16, HPV-31, DENV1, DENV2, H5N1, HTLV-1, HSV-1, HSV-2, HVC, HVB, and hRSV (Table 3) *in vitro* and/or *in vivo*, we are interested in LMWH. These Hep-sensitive viruses use HSPGs as receptors (Table 1). Some studies have shown that Hep stimulates the biosynthesis of HS 2- to 3-fold on cell surfaces (endothelial cells and fibroblasts) (see Section 5).

Another molecule that has shown antiviral properties against viruses, including SARS-CoV-2 and hRSV, is xylitol (see Table 3). Indeed, as anticipated from a previous article [35], xylitol completely inhibited SARS-CoV-2 in an *in vitro* study conducted by Bansal et al. [72], where xylitol was used as a placebo. In addition to a patent filed by a French company demonstrating that D-xylose (a direct metabolite of xylitol) significantly stimulates the biosynthesis of HS [103], an *in vitro* study conducted by Johnston and Keller [104] showed that incubation of SV3T3 cells with β-xylosides resulted in a 10-fold increase in the biosynthesis of HS chains.

Schmidt et al. [70] showed the importance of HS chains in controlling the integrity of the endothelial glycocalyx and on inflammation and lung injury. The stimulation properties of HS biosynthesis, which are shared by Hep [95–97,99], D-xylose [103] and other β-xylosides, logically link the anti-inflammatory properties to the most commonly used of these compounds: Hep [105,106]. The anticoagulation properties of Hep [107,108] also are linked to the anticoagulation properties of HS chains [109,110].

The hypothesis that the viruses listed in the Table 1 would be attached to the HS/CS attachment sites (*i.e.*, on the Ser residues intended to receive D-xylose for initiation of the biosynthesis of HS/CS or DS chains) attributes, in fact, the inhibitory properties of viral attachment to any compound that stimulates the biosynthesis of HS chains.

However, care must be taken to ensure that HS stimulated by Hep, D-xylose or other xylosides are not (only) soluble HS chains. Indeed, if these HS chains are only soluble, inhibition of the virus by Hep or xylosides could be attributed to the action of these additional soluble HSs based on the assumption that soluble HSs could act as viral decoys. However, it is the HS secreted by the Golgi apparatuses of endothelial and fibroblast cells whose biosynthesis is stimulated by Hep [95–97]. Moreover, the question of whether D-xylose stimulates the biosynthesis of only soluble HS is even more crucial. Indeed, uridine diphosphate xylose (UDP-xyl), which is used as a substrate by the enzyme xylosyltransferase I to initiate the biosynthesis of HS chains, comes from GlcA and not xylose [111]. However, although the patent (WO1999024009A1 - see the pdf) [103] from the large French company was on skin cells, it has been well specified that the PGs and GAGs whose D-xylose significantly stimulates HS biosynthesis are secreted by keranocytes and fibroblasts. In addition, the 1992 works of Fransson and colleagues on skin fibroblast cells revealed that the treatment of cells with Hep resulted in a decrease in the deposition of PGs in the cell pericellular matrix and an increase in cell-associated PGs [98]. These authors also mentioned that their results suggested that exogenous xylosides enter the ER and are transported into the Golgi apparatus where the biosynthesis of HS takes place [98]. It is worth mentioning that the HS chains formed by β-xylosides (modified xylose) are not natural HS chains; therefore, their effects on the cell may be different from Hep- (which shares the same linkage region as HS/CS/DS) and D-xylose-stimulated biosynthesized HS chains. However, by occupying HS attachment sites on core proteins, Hep and D-xylose can inhibit the attachment of viruses (from our hypothesis). Thus, several polysaccharides containing D-xylose could inhibit the attachment of viruses *in vitro* (see Table 3). As the effects of certain LMWHs are immediate (a few minutes) on inflammation, coagulation and inhibition of the attachment of viruses, it is reasonable to question whether these properties are solely due to the fact that Hep stimulates HS biosynthesis on the cell surface. The answer to this question was provided by Nader et al. [95]. They reported in the summary of their work that the increase in HS biosynthesis began immediately after the exposure of the cells to Hep. In addition, Trindale et al. [100] reported that their results indicated that exogenous Hep also binds directly at the HS attachment sites on the core proteins at the cell surface. This is logical, since endogenous Hep occurs in the human body (produced by mast cells) at the same binding sites as HS chains. Thus, it can also help in the inhibition of viral entry, and its (endogenous Hep) production can probably be enhanced in the body by natural exogenous D-xylose and exogenous Hep. This is also the case for the other sulfated GAGs, CS and DS, which share the same linkage region (Xyl-Gal-Gal) with Hep and HS. Stimulation of the biosynthesis and secretion of these 2 types of sulfated GAGs (CS/DS) by the D-xylose and β-xylosides has been proven in aligning studies conducted in 1974 and 1978 by Schwartz et al. [112–114]. Schwartz concluded in one of these studies that the lack of carbohydrates alters the CS [112].

We have seen that PTMs of Ser residues of the core proteins on which the biosynthesis of HS is initiated are proven O-glycosylation sites and that these positions are listed as such in databases (see Section 4.2). However, above all, we saw that, in an *in vitro* study, Hudák et al. [6] demonstrated indirectly that the spike glycoprotein of SARS-CoV-2 binds well to HS/CS attachment sites (*i.e.*, on the Ser residues of the core proteins intended to receive D-xylose for initiating the biosynthesis of HS/CS chains) (see Section 4.2).

In addition, Bourgeois et al. [79] noticed in 1996 in an *in vitro* study on hRSV with a molecule with a Hep-like structure, that coadministration of Hep and the virus were competed to attach themselves to the free space. Moreover, treatment of the cells with Hep resulted in 100% inhibition of the virus [79]. In 2014, Milewska et al. [10] noticed in an *in vitro* study that in the presence of soluble HS, the adhesion of HCoV-NL63 was completely inhibited. Taken together, the similarities between Hep and HS [97] (same linkage region, Hep regions in the HS structure, *etc.*) showed that Hep enters the cell and is transported into the Golgi apparatus where the biosynthesis of HS secreted by the cell takes place [95,96,98]. Additionally, due to the properties of Hep and other xylosides on the stimulation of HS secreted by the cell, the diversity of viruses involved in *in vitro* studies with similar results and our hypothesis, it is logical to wonder whether the results obtained by Milewska et al. [10] would not be associated with stimulation of the biosynthesis of HS secreted by cells.

The processes summarized in Fig. 2 show why the promotion of HS biosynthesis is a more strategic antiviral option based on our hypothesis.

Case (a) corresponds to the situation where there is enough D-xylose (exogenous D-xylose molecules or D-xylose coming from xylosyltransferase I enzyme) to initiate the biosynthesis of HS chains in the Golgi apparatus. This situation leads to the inhibition of virus attachment to the core protein (step (a)). Case (b) corresponds to the situation where some HS/CS attachment sites (*i.e.*, Ser residues intended to receive the D-xylose molecules for initiating HS/CS chains) are free due to a lack of D-xylose. This situation promotes viral infection because the core protein at the cell surface has free Ser residues (which were intended for HS biosynthesis) acting as viral receptors *via* O-linked glycosylation (step (1)), followed by viral entry and viral replication (steps (2) to (7)). In case (c), the viruses in the Golgi apparatus can be glycosylated at the HS/CS attachment sites (*i.e.*, Ser residues that were intended for HS biosynthesis). This case leads to a reduction in the number of HS chains at the cell surface (step (8)).

7. Conclusions

As summarized in Fig. 3, we conclude that (i) LMWHs and β-xylosides upregulate HS biosynthesis at the cell surface; (ii) Ser residues, which are HS chain receptors on core proteins, are O-glycosylation sites for viruses; (iii) HS biosynthesis blocks (*via* D-xylose) the Ser residues utilized by viruses as receptors at the cell surface; (iv) HS biosynthesis induces HPSE reduction at the cell surface; (v) reduced HPSE minimizes shear-induced shedding of syndecans, thereby sustaining glycocalyx

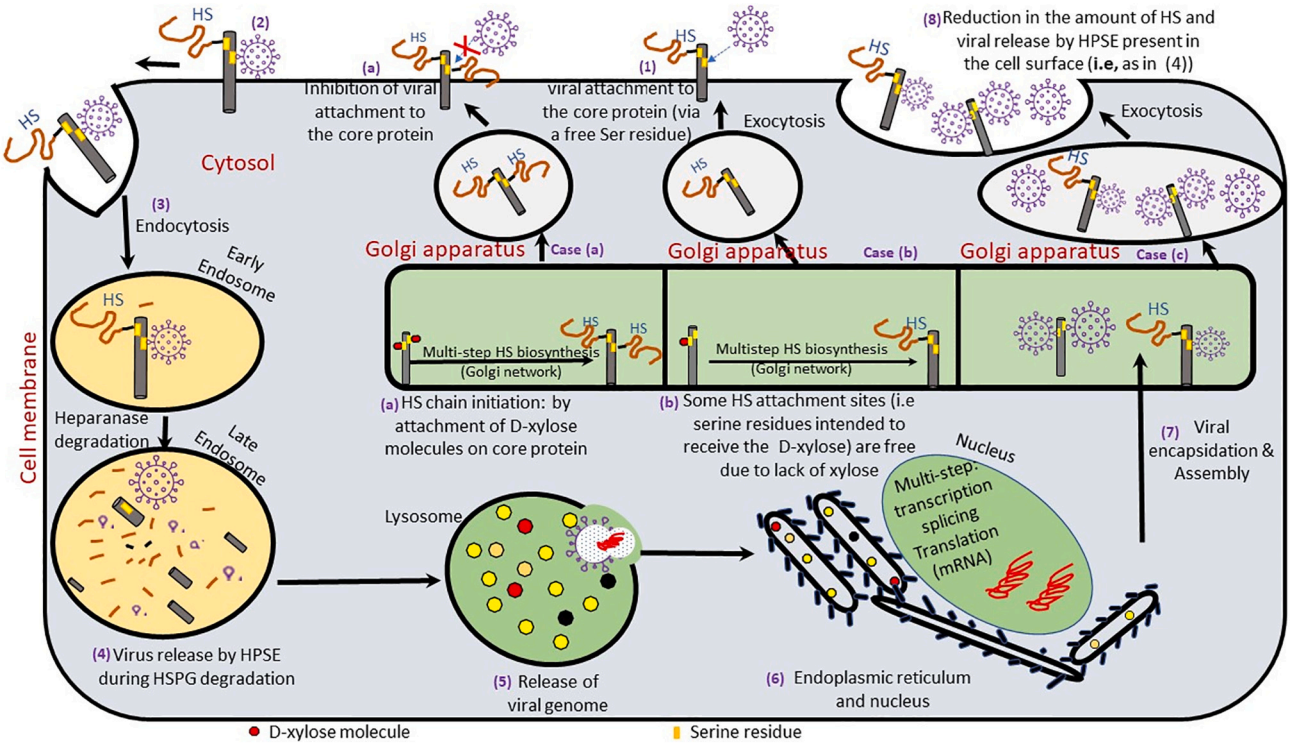

Fig. 2. Heparan sulfate (HS) biosynthesis and viral attachment.

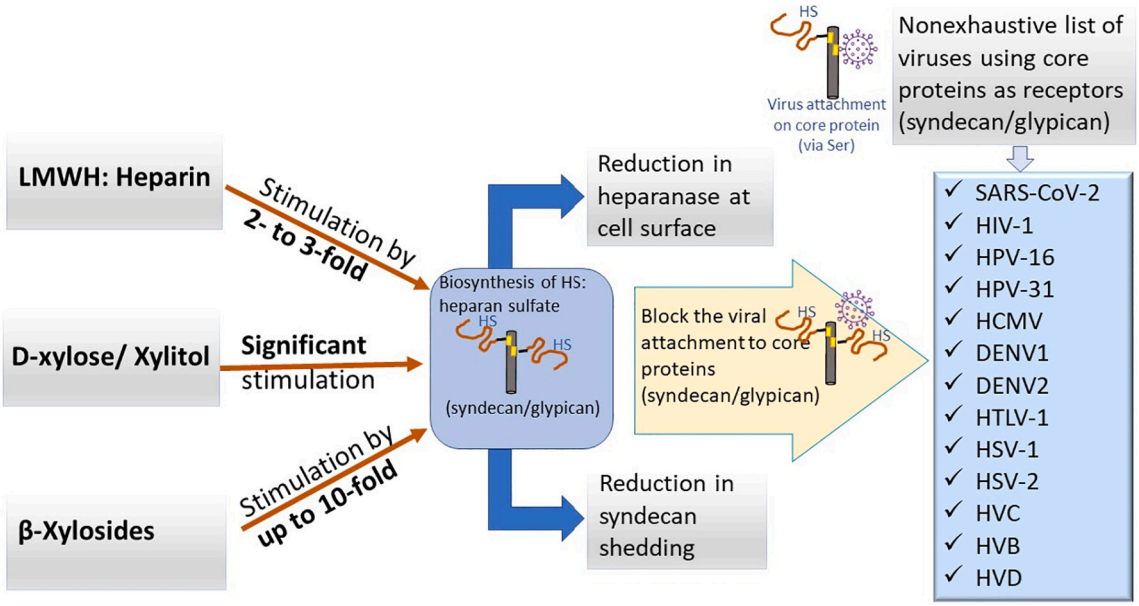

Fig. 3. Stimulation of the biosynthesis of heparan sulfate (HS) by LMWHs and xylosides blocks viral attachment to core proteins.

integrity and reducing inflammation; and (vi) antiviral strategies should focus on HS biosynthesis.

8. Limitations

There are some limitations to this article. For instance, several relevant studies on viruses that include SARS-CoV-2, HIV-1, and HVC were not included. In addition, the provided list of viruses that use the core proteins as receptors was not exhaustive. Furthermore, some studies have also demonstrated that xylitol (*via* D-xylose) decreased the number of cell adhesion molecules (CAMs) on the cell surface up to 10-fold. This implies that stimulation of HS biosynthesis will significantly reduce the internalization of viruses that use CAMs as receptors, and this possibility has not been addressed.

Declaration of competing interest

The authors declare that there are no conflicts of interest.

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Ethical considerations

This is a review article. Hence, informed consent and ethics committee approvals as well as compliance with the Declaration of Helsinki are not applicable.

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