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Natural small molecules as inhibitors of coronavirus lipid-dependent attachment to host cells: a possible strategy for reducing SARS-COV-2 infectivity?

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Abstract: *Background:* Viral infectivity depends on interactions between components of the host cell plasma membrane and the virus envelope. Here we review strategies that could help stem the advance of the SARS-COV-2 epidemic. *Methods and Results:* We focus on the role of lipid structures, such as lipid rafts and cholesterol, involved in the process, mediated by endocytosis, by which viruses attach to and infect cells. Previous studies have shown that many naturally derived substances, such as cyclodextrin and sterols, could reduce the infectivity of many types of viruses, including the coronavirus family, through interference with lipid-dependent attachment to human host cells. *Conclusions:* Certain molecules prove able to reduce the infectivity of some coronaviruses, possibly by inhibiting viral lipid-dependent attachment to host cells. More research into these molecules and methods would be worthwhile as it could provide insights the mechanism of transmission of SARS-COV-2 and, into how they could become a basis for new antiviral strategies.

Key words: Coronavirus, SARS-COV-2, lipid raft, cholesterol, phytosterol

Introduction

Glycoproteins S (spike proteins) of the new coronavirus (SARS-CoV-2) bind to the Angiotensin-Converting Enzyme 2 (ACE2) receptors on human respiratory epithelial cells. During attachment, the S glycoprotein divides into S1 and S2 subunits. S1 contains the receptor-binding domain by which the coronavirus binds to the peptidase domain of the ACE2 receptor. S2 intervenes later, during fusion of the plasma membranes (1). Whole genome sequencing analysis of 104 isolates of the virus causing SARS-COV-2 in patients from different locations showed 99.9% homology, without significant mutations. Almost 80% homology with the SARS-CoV genome and more than 90% with the bat coronavirus genome were also confirmed (2,3). Indeed, the new coronavirus causes a disease that has SARS-CoV-like symptoms. In particular, studies suggest that the SARS-COV-2 receptor that recognizes ACE2 on the target cell membrane is similar to that of

SARS-CoV. SARS-COV-2 nucleocapsid protein has an amino acid sequence identity of almost 90% with SARS-CoV (4).

Most studies on the new SARS-COV-2 are based on previous research carried out on other human coronaviruses in order to identify molecular targets that could be blocked to inhibit entry of the virus into the cell. Coronaviruses are a class of viruses with a long single positive RNA molecule (27-30 kb) and a lipid envelope that requires a plasma membrane fusion process mediated by endocytosis, a mechanism in which cholesterol and lipid rafts play a fundamental role in the early stage of infection of a cell (5,6).

Materials And Methods

This qualitative review is based on data from original documents and reviews. The most pertinent studies on cholesterol and lipid rafts are summarized and interpreted to highlight the role of these membrane molecules in coronavirus infectivity, with a view to their possible consideration in new studies as targets for reducing human infectivity of SARS-COV-2.

We conducted an electronic search in Medline, PubMed and Scopus using different combinations of the search terms "virus", "SARS-COV-2", "coronavirus", "lipid raft", "sterol OR phytosterol" and "cholesterol". This allowed us to link the most recent studies on the infectivity mechanism of SARS-COV-2 with past studies on the role of membrane lipids in the attachment of viruses to host cells. We included studies concerned with the infectivity of types of virus that exploit lipid components of the host cell. The references of all articles were scanned to retrieve any relevant information.

Results

Cholesterol rafts and viral attachment

Cholesterol on the target cell is important for SARS-CoV infection. In the initial stages of a SARS-CoV and Human Parainfluenza virus infection, cholesterol and lipid membrane rafts play a fundamental role in viral entry into the cell (7). The virus attacks these surface molecules on the host cell in specific areas of the plasma membrane characterized by lipid rafts (5). Certain cholesterol-rich microdomains facilitate interaction between the spike protein and its ACE2 receptor (8). SARS-COV-2 is a member of a virus family with a lipid envelope that fuses with the host cell through endocytosis, internalizing its components in the cell (5).

Lipid rafts are important plasma membrane areas for the endocytosis process (5,6), for instance in the early stages of internalization of coronaviruses (9). For example, lipid rafts are involved in the entry of infectious bronchitis coronaviruses. The murine hepatitis coronavirus also requires specific interactions between its spike proteins and lipid rafts on host cells (10,11). This is confirmed by other studies showing that the infectivity of viruses, including coronaviruses, is stimulated by homeostatic control of cholesterol and regulation of fatty acid metabolism (12). *In vitro* experiments show that the sensitivity of the virus to fusion with the host cell membrane increased by cholesterol supplements. For example, cholesterol-supplemented mouse fibroblasts showed increased susceptibility to fusion with murine hepatitis virus (13). There is no significant co-localization of ACE2 with lipid rafts in the plasma membrane of cell models for SARS-CoV infection studies (14). In any case, in lipid raft areas there are also caveolins, clathrins and dynamin, molecules that could have a fundamental role in the internalization of viruses. Internalization mechanisms that depend strictly on these molecules and on the presence of lipid rafts have been described for the simian virus (SV40) (6).

Molecular inhibitors of virus lipid-dependent attachment

Macromolecules such as methyl-β-cyclodextrin (MβCD) and other compounds with depletive cholesterol activity have been used to inhibit attachment of coronaviruses to host cells (15). The lipophilic core allows the interaction of these molecules with lipid rafts (Figure 1). Studied for their antiviral activity, these non-toxic macromolecules mimic attack sites for the enveloped virus, competing with host cell attack sites (16,17). Longer exposure to high MβCD concentrations may also lead to redistribution of cholesterol between raft and non-raft membrane regions (18).

In vitro cell models expressing the ACE2 membrane protein have shown that depletion of cholesterol by MβCD halved the number of bonds with viral S glycoproteins (8). The molecule not only affects cholesterol levels but also expression of the ACE2 receptor. Some studies showed that MβCD treatment slightly and dose-dependently reduced expression of ACE2 in the cell membrane, also reducing the infectivity of coronaviruses, such as SARS-CoV (14).

Other lipophilic molecules, classified as phytosterols, readily interact with the molecules of lipid rafts. This interaction can lead to membrane cholesterol reduction or destabilization of its structure (Figure 2). It could also influence biochemical signaling activities downstream of the rafts (20). These natural molecules are similar in structure to cholesterol. One, extracted from the root of *Aerva lanata*, affects HIV infectivity (21). Other plant sterols, such as β-sitosterol, reduce the probability of HIV and HBV infectivity (22). Řezanka et al. described the antiviral activity of sterols and triterpenoids, molecules which form the basis of many new synthetic drugs aimed at reducing the infectivity of viruses (23). It is well documented that regular phytosterol intake can reduce LDL cholesterol by around 10% (24,25). Betulinic acid is a phytosterol that also has lipophilic properties. Like triterpenes, this compound has a structure similar to cholesterol (Figure 2). It may therefore compete with cholesterol, replacing it in plasma membranes, or it may link to the virus instead of raft cholesterol, acting as a soluble competitor (20). Liposomes are widely used for studying lipid rafts (26). These models have demonstrated that treatment of virus-infected cells with polyunsaturated liposomes has effective antiviral activity in HCV, HIV and HBV infection, reducing levels of cell- and virus-associated cholesterol (27). This treatment could reduce attachment of viruses to host cells.

Discussion and Conclusions

The role of lipids in viral infections suggests cues for understanding the recent SARS-COV-2 infection. Targeting host lipids is already being studied as an antiviral strategy and could have various applications (28).

Cyclodextrins and phytosterols could have health benefits, such as reduction of blood cholesterol levels, and are used to prevent and reduce the risk of coronary disease, to reduce inflammation, to induce apoptosis in cancer cells and to treat viral infections. Today these natural compounds are also available in different forms as supplements. The aim of the present review was to offer a panorama of the role of these molecules in one of the many mechanisms involved in the entry of viruses into host cells. These molecules and their antiviral properties should be borne in mind in double

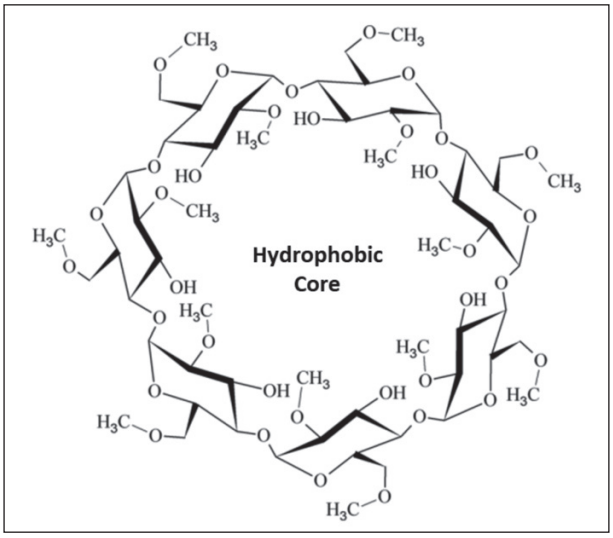

Figure 1. Molecular structure of a methyl-β-cyclodextrin. Adapted from Fenyvesi et al. (19)

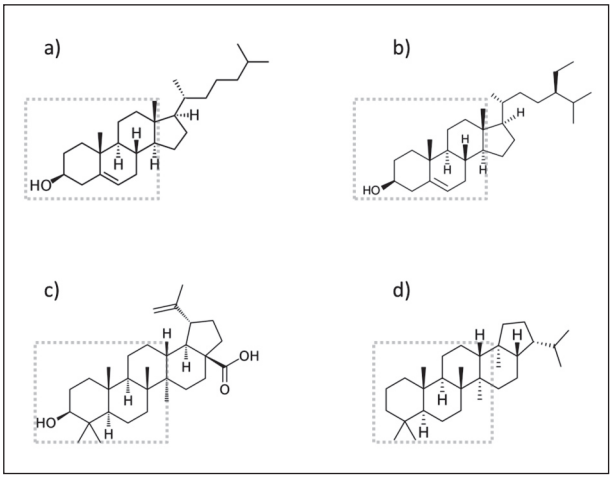

Figure 2. Molecular structure of cholesterol (a), β-sitosterol (b), betulinic acid (c) and hopane, an example of a pentacyclic triterpene (d). Similarities in core structure are marked by dashed line.

blind trials concerning the SARS-COV-2 pandemic, testing them in a cohort of volunteers and patients and reporting any evidence of their ability to reduce viral infectivity.

Conflict of interest: The author declares that he has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article

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