ANTIVIRAL ACTIVITIES OF POLYSACCHARIDES FROM NATURAL SOURCES

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ABSTRACT: The ever increasing resistance of human pathogens to current anti-infective agents is a serious medical problem, leading to the need to develop novel antibiotic prototype molecules. In the case of viruses, the search for antiviral agents involves additional difficulties, particularly due to the nature of the infectious viral agents. Thus, many compounds that may cause the death of viruses are also very likely to injure the host cell that harbours them. Natural products are increasingly appreciated as leads for drug discovery and development. Screening studies have been carried out in order to find antiviral agents from natural sources, and the occurrence of antiviral activity in extracts of plants, marine organisms and fungi is frequent. The evidence indicates that there may be numerous potentially useful antiviral phytochemicals in nature, waiting to be evaluated and exploited. In addition, other plants, not previously utilized medicinally, may also reveal antivirals. Among natural antiviral agents, recent investigations have reconsidered the interest of phyto-polysaccharides, which act as potent inhibitors of different viruses. This chapter will illustrate a variety of antiviral polysaccharides from natural sources since 1990, with the aim of making this matter more accessible to drug development

INTRODUCTION

Infectious diseases account for one-third of all deaths worldwide. Although the last decade has yielded significant advances in the treatment of infectious diseases, new therapies for viral, fungal, bacterial and parasitic infections are needed [1].

Viruses are responsible for a large proportion of the morbidity and mortality experienced worldwide. These infectious agents consist of a core genome of nucleic acid, DNA or RNA (nucleoid) contained in a protein shell (capsid) and sometimes surrounded by a lipoprotein membrane (envelope). Some genera of viruses are known to cause human infections and include the following: adeno, pox and herpesviruses:

herpes simplex virus type 1 and 2, varicella-zoster, cytomegalovirus, Epstein-Barr virus; papillomaviruses (papillomas, cancer); parvoviruses (erythema infectiosum); DNA-viruses (e.g., hepatitis B); (+/-) RNA viruses: reo- and rotavirus; (-) RNA viruses: influenza, parainfluenza, measles, respiratory syncytial, vesicular stomatitis, rabies virus and togaviruses (rubella, yellow fever); retroviruses (e.g., human immunodeficiency virus, the causative agent of acquired immunodeficiency syndrome); and other several RNA viruses such as arenaviruses (lymphocytic choriomeningitis), bunyaviruses (encephalitis), coronaviruses (upper respiratory infections) and picornaviruses (poliomyelitis, diarrhoea). Most successful anti-infective agents inhibit essential steps in metabolic processes required by the pathogen but absent in the host. However, viruses cannot replicate independently. They must enter cells and use the cellular energy-generating, DNA- or RNA-replicating and protein-synthesizing pathways of the host to achieve viral replication. For this reason, in the case of viruses the search for antiviral agents is very hard since metabolic differences are not available: viruses utilize the host's protein synthesis leaving only some aspects of nucleic acid synthesis and macromolecule processing distinct from the host's metabolism.

The identification of a retrovirus, human immunodeficiency virus, as the causative agent of acquired immunodeficiency syndrome, the steadily increasing incidence of various viral infections caused by viruses in immunodeficient patients, e,g., herpes simplex virus, varicella-zoster virus, cytomegalovirus and Epstein-Barr virus, the increasing evidence for the pathogenic role of a number of viruses, e.g., human papilloma virus in the pathogenesis of primary hepatocellular carcinoma, and the socioeconomic impact of viral infections of the respiratory (influenza, adeno-, corona- and rhinoviruses) and gastrointestinal tract (rotaviruses) have all been important factors in boosting the search for new antiviral agents and new modalities of antiviral chemotherapy. Although many compounds with potent antiviral activity in cell cultures and in experimental animals have been detected, at present only several molecules and α-interferon have been approved by the health authorities for therapy of viral infections in humans. Among these antiviral substances are several natural compounds isolated from plants used in traditional medicine, including polysaccharides [2-4].

Polysaccharides (PS) constitute a structurally diverse class of biological macromolecules with a wide range of physicochemical properties, which are the basis for different applications in the broad field of pharmacy and medicine [5-8]. Several hundred natural PS are currently known and provide one of the richest and oldest reservoirs of structurally and functionally diverse biopolymers. PS constitute important members of the family of industrial water-soluble polymers. Besides the classical applications of these biopolymers in industry and pharmaceutical practice, the relatively new field of pharmacologically active polymers will be discussed. PS from plants and other natural sources have long been known to exert antitumour, immunomodulatory, anticoagulant and other types of biological activity, including antiviral activity. The importance of the plant kingdom as a source of new antiviral substances will be illustrated by presenting a survey on natural-derived antiviral PS.

In recent years, natural and synthetic polymers of a carbohydrate nature were proved to be selective inhibitors of several enveloped viruses, including such human pathogens as human immunodeficiency virus, herpes simplex virus, human cytomegalovirus, influenza virus and respiratory syncytial virus. Recent efforts have been directed at the development of polysaccharidic molecules as selective inhibitors of different classes of viruses [9,10]. In this search, the following molecular targets were envisaged: for DNA viruses in general, the viral DNA polymerase; for herpes simplex virus and varicella-zoster virus, the viral DNA polymerase via a specific phosphorylation by the viral 2'-deoxythymidine kinase; for (+/-) RNA and (-) RNA viruses, S-adenosylhomocysteine hydrolase, a key enzyme in transmethylation reactions required for the maturation of viral mRNA; for retroviruses, reverse transcriptase as an initiator of virus replication and/or cell transformation; and for several enveloped viruses (e.g., retro-, herpes- and rhabdoviruses), virus adsorption to the outer cell membrane. This review deals with antiviral PS compiled from several sources during the past decade. Under the respective titles, we offer some brief descriptions of the most important global viral infections that are caused either by individual viruses or by groups of related viruses. They are restricted to those generally considered important in human and veterinary medicine and about which reports on active antiviral PS are recorded in the literature.

HUMAN IMMUNODEFICIENCY VIRUS

Acquired immunodeficiency syndrome (AIDS) is a pandemic immunosuppressive disease which results in life-threatening opportunistic infections and malignancies. In 1996, the number of AIDS infections was 23 million and 1.5 million people (half of them in Africa) were killed by this disease worldwide. In order to combat human immunodeficiency virus (HIV), the causative agent of AIDS, enormous amounts of time and money have been dedicated to research into compounds which can be developed as therapeutic agents. HIV is a lentivirus which replicates within critical cells of the immune system, particularly CD4 T-cells and monocyte/macrophages, leading to a progressive loss of helper T-cells and profound immunosuppression. This condition is known as AIDS. Without treatment, the CD4 T-lymphocytes of an infected patient are reduced markedly so that the patient is susceptible to a wide range of opportunistic infections, and ultimately dies. Since this retrovirus, designated HIV, has been clearly identified as the primary cause of this disease, numerous compounds, also including plant-derived substances, have been evaluated for their inhibitory effects on HIV replication *in vitro*. Furthermore, many plant products are being used by patients with AIDS in some countries without any scientific proof that they possess anti-HIV activity.

Several reviews on natural products for the chemotherapy of HIV infections have been recorded in the literature [11-19]. Carbohydrates were among the natural molecules capable of treating HIV infection. Various PS, including sulphated PS, have been found to be anti-HIV active substances of many antivirally active plant extracts, all of which are medicinal plants used in traditional medicine as anti-infectives. For example, a PS (MAR-10) was isolated from the aqueous extract of the plant *Hyssop officinalis* L., which was found to inhibit HIV-1 replication as demonstrated by the inhibition of HIV-1 p24 antigen and syncytia formation [20]. From *Aspalathos linearis* Burm., a popular medicinal plant in South Africa, Europe and Japan, a PS with strong anti-HIV activity was isolated [21,22]. This compound almost completely inhibited the binding of HIV-1 to MT-4 cells. The major sugar components of this purified PS were found to be glucose, galactose, mannose and xylose. Premanathan *et al.* [23,24] investigated the PS extracted from *Rizophora apiculata* Blume and *Rizophora mucronata* Poir. in cell culture systems

for their activity against human and simian immunodeficiency viruses (SIV). Both compounds inhibited HIV-1, HIV-2 or SIV strains in various cell cultures and assay systems. It was found that these PS consisted mainly of neutral sugars and uronic acids.

In a screening programe of Thai plants for anti-HIV activity, it was found that 114 extracts of 81 species belonging to 32 families showed activity [25]. Of the effective samples, the extracts of *Merremia peltata* (L.) Merrill were the most active. Most of the anti-HIV agents were found to be anionic PS. Furthermore, sulphated derivatives of paramylon, a (1-3)-β-D-glucan from *Euglena gracilis* Klebs. significantly inhibited the cytopathic effect of HIV-1 and HIV-2 [26], while two naturally occurring PS isolated from the tropical climbing shrub *Ipomoea cairica* (L.) Sweet were found to strongly inhibit replication of HIV-1 *in vitro* [27].

According to De Clercq [28], the replicative cycle of HIV comprises several steps that could be considered adequate targets for chemotherapeutical intervention: virus entry, viral adsorption, virus-cell fusion, reverse (RNA → DNA) transcription, proviral DNA integration, viral (DNA → RNA) transcription (transactivation), viral (mRNA → protein) translation, virus release, viral assembly, budding and maturation. The most notable difference between HIV replication and other retroviruses is the intricate control of HIV gene expression by viral and cellular factors [29]. Possible mechanisms by which HIV kills infected cells include the formulation of multinucleate syncytia, cytopathic components within the virions themselves, and interaction between viral envelope proteins and the CD4 molecule on the cell surface. Three HIV enzymes are essential to the life cycle of the virus: HIV reverse transcriptase is crucial for viral replication; HIV protease processes viral polyproteins into functional enzymes and structural proteins, thereby facilitating maturation and infectivity of the virion particles; HIV integrase mediates HIV integration into the host chromosome. The chemotherapeutic strategies have therefore been focused on the development of inhibitors of these retroviral enzymes. Recently, it has been reported that biological membranes arising from HIV-induced cell fusion, as well as syncytium formation between infected and non-infected cells are interesting approaches in research for a new HIV therapy [30].

Natural sulphated PS such as dextran sulphate, pentosan sulphate, chondroitin sulphate and heparin have long been recognized as effective anti-HIV agents [31-35]. These compounds target the interaction between

the viral envelope glycoprotein gp120 and the CD4 receptor and, as a consequence, they inhibit not only virus adsorption to the cells but also virus-induced syncytium (giant cells) formation [36-42]. The inhibitory effect of dextran sulphate and its congeners on viral binding, viral replication and syncytium formation appears to be mediated by a specific interaction with the V3 region of gp120. In addition, sulphated PS may also directly interfere with the binding of HIV particles to the heparin sulphate proteoglycan of the cell surface, whether or not this process occurs independently of, or cooperatively with, the viral envelope CD4 receptor interaction.

This class of antiviral agents exhibits several unique properties that are not shared by other currently known antiviral agents: remarkable broad-spectrum antiviral activity against HIV-1, HIV-2 and a series of other enveloped viruses; the ability to inhibit syncytium formation between HIV-infected and normal CD4 T-lymphocytes, a mechanism that drastically enhances HIV infectivity and a low induction of viral drug-resistance [43]. It should be noted that sulphated PS owe their anti-HIV activity to the presence of the sulphate groups, which in turn are responsible for the inhibition of virus-cell binding. Dextran is a high-molecular-weight PS consisting predominantly of α (1 $\rightarrow$ 6) linked D-glucose units. Dextrans can differ in chain length and in degree of branching. The branching occurs via α (1 $\rightarrow$ 3) and α (1 $\rightarrow$ 4) branch points. Dextran fractions of different average molecular weight were obtained by partial hydrolysis. These different fractions were sulphated to obtain dextran sulphates of different molecular weight.

Busso and Resnick [44] investigated the effects of three molecular weight ranges of dextran sulphate on five different HIV isolates. The higher molecular weight range of dextran sulphate showed the most potent activity as assessed by a quantitative syncytium formation assay. However, dextran sulphate is poorly adsorbed after oral administration and upon intravenous administration produces thrombocytopenia, so that there is little, if any, evidence for the *in vivo* efficacy of this compound and more generally of sulphated PS against HIV infection [45]. However, double-blind placebo-controlled clinical pilot trials in advanced HIV disease are being conducted with these compounds [46].

In summary, these compounds offer great promise as candidate drugs for the chemotherapy of HIV infections, and novel sulphated PS which

have proved to be potent and selective inhibitors of HIV *in vitro* are being chemically synthesized [47-52].

On the other hand, although several groups have reported that sulphated PS are potent and selective inhibitors of HIV, their therapeutic application is however limited by their anticoagulant activity. In view of possible improvements in therapeutic potential, a number of heparin derivatives with reduced anticoagulant activity were studied for their inhibitory activity in an HIV-dependent syncytium formation assay [53]. The most pronounced anti-HIV activity was observed with 30% succinylated standard heparin and 100% succinylated low molecular weight heparin. Similarly, and in order to increase the ratio of anti-HIV activity to anticoagulant activity, selectively substituted sulphated PS with OH and/or COOH groups were chemically prepared [54,55]. This group comprises a family of heterogeneous carbohydrates composed of long, unbranched PS modified, for example, by sulphations and acetylations. The acetylated derivatives are more active as inhibitors of HIV-induced giant-cell formation. Furthermore, several soluble derivatized dextrans with different percentages of carbomethyl, benzylamide and sulphonate/sulphate groups were also evaluated for possible inhibitory effects on HIV-1 infection, and from the results obtained, their use as anti-HIV therapeutic agents can be proposed [56].

Other natural sources of anti-HIV sulphated PS include algae and other marine organisms. Sulphated PS have been found in a variety of marine animals, plants and microorganisms [57,58]. Aqueous extracts of many marine invertebrates have exhibited some activity in the National Cancer Institute's primary screen for anti-HIV cytopathicity [59]. Carrageenans and fucoidan are sulphated PS extracted from red seaweed and brown algae, which have shown potent inhibitory activity against different viruses including HIV. For example, fucoidan, a complex sulphated PS from the alga *Fucus vesiculosus* L. was found to inhibit HIV *in vitro*, presumably through a direct interaction of the PS with the HIV binding site of the target cells [60]. The aqueous-soluble extracts from this alga, a common littoral alga of the coast of the Northern Atlantic, the Pacific Ocean and the Baltic Sea, inhibited the activity of HIV reverse transcriptase enzyme as well as HIV-induced syncytium formation. Béress *et al.* [61] reported a new procedure for the isolation of the anti-HIV PS from this marine alga.

A novel sulphated PS named calcium spirulan has been isolated as an antiviral principle from *Spirulina platensis* (Nordst.) Geitl., a blue-green

alga growing in some African and Central and South American lakes rich in salts [62-65]. Chemical analyses of calcium spirulan suggest that it contains rhamnose, ribose, mannose, fructose, galactose, xylose, glucose, glucuronic acid and galacturonic acid. Other sulphated PS from marine sources have demonstrated antiviral activities against HIV, blocking viral replication in cell culture without any toxicity to the host cells. Examples include sulphated cell-wall PS from the Mediterranean red alga *Asparagopsis armata* Harvey [66], from the marine microalga *Cochlodinium polykrikoides* Blooms [67], fucose, the main component sugar of the PS isolated from the brown alga *Sargasum horneri* (Turner) C. Agardh [68] and *Sargasum muticum* (Yendo) Fensholt. [69], sulphated PS from Brazilian marine algae such as *Laminaria abyssalis* AB Joly & EC Oliveira [70], and from tropical marine algae of the Codiaceae family [71], and new sulphated β-galactans from the marine clam species *Meretrix petechialis* Lamarck [72]. Other natural sources of anti-HIV sulphated PS include fungi such as *Ganoderma lucidum* (Fr.) Karst. [73] and *Lentinus edodes* (Lem.) mycelia [74].

Inhibition of HIV-reverse transcriptases (HIV-RT) is currently considered a useful approach in the prophylaxis and intervention of AIDS and natural products have not as yet been extensively explored as inhibitors of this enzyme [75,76]. Furthermore, the mechanisms of action of sulphated PS may not only be due to the inhibition of virus cell adhesion, but also to the inhibition of HIV-RT [13,15].

HERPES SIMPLEX VIRUS

Herpes infections are ubiquitous. Approximately, 16-35%, 40-80% and >90% of the population are sero-positive for, or infected by herpes simplex virus type I (HSV-1), herpes simplex virus type II (HSV-2) and varicella-zoster virus, respectively. More alarmingly, over the past decade, the incidence and severity of infections caused by HSV have increased due to the growth in number of immunocompromised patients produced by aggressive chemotherapy regimens, expanded organ transplantation and a greater occurrence of HIV infections. Unfortunately, prolonged therapies with acyclovir, the most successful antiherpetic drug, have resulted in some undesirable complications and also induced the emergence of drug-resistant viruses. With this change in disease pattern,

and the increase in drug use frequency, acyclovir-resistant HSV infections have emerged.

Infections with HSV range from simple cold sores and fever blisters to severe central nervous system disorders. Development of effective antiviral medications has made prompt recognition important in primary care practice. Appropiate therapy can significantly reduce both the medical and psychosocial ramifications of herpes infections and can greatly improve the quality of life of many patients [77]. Thus, there is an urgent need for novel anti-HSV agents, especially those with a different mode of action from acyclovir. For the past decades, in addition to a variety of synthetic antiviral drugs with different molecular targets, a large number of phytochemicals have been recognized to control infections caused by viruses belonging to the Herpesviridae family. Ethnopharmacological screenings of medicinal plants from all over the world have led to the selection of several extracts which are active against herpes viruses.

Among natural antiviral agents, recent investigations have revived the interest in phyto-PS, which act as potent inhibitors of different enveloped viruses, including members of Herpesviridae. For example, a neutral PS extracted from *Sclerotium glucanicum* Halleck., namely scleroglucan, revealed promising antiviral activity [78]. These PS are composed of a β-1,3-linked glucopyranose backbone with single β-1,6-linked glucopyranosyl branches every third subunit. The blockage on HSV infection takes place during the very early phases of the viral multiplication cycle, since the greatest inhibitory effect occurred when it was added during the attachment step.

As a result of the intensive search for antiviral substances from medicinal plants, antiviral activity against HSV was found in extracts from *Cedrela tubiflora* Bertoni. leaves [79], from *Prunella vulgaris* L., a perennial plant commonly found in China, the British Isles and Europe [80] and from *Trichilia glabra* L. leaves [81]. Phytochemical studies indicate that these plants contain anionic PS as active constituents which may inhibit HSV by competing for cell receptors as well as by some unknown mechanisms after the virus has penetrated the cells. Furthermore, the *in vitro* antiviral activity demonstrated by extracts of the medicinal plant *Achyrocline flaccida* (Weinm.) D.C. on HSV-1 is exerted early during viral replication, essentially during viral adsorption to host

cells [82]. A bioguided purification process indicated that negatively charged PS were responsible for this antiviral activity.

Among natural antiviral agents of a carbohydrate nature, recent investigations have also revived interest in sulphated phyto-PS such as carrageenans and fucoidan from seaweed. Sea algae represent a very interesting source of potential antiviral compounds, particularly the water-soluble sulphated PS which are abundant constituents of the cell wall. These compounds act as potent inhibitors of different enveloped viruses, including members of Herpesviridae, and their activity is linked to the anionic features of the molecule which hinder the attachment of viral particles to host cells. For example, carrageenans are sulphated galactans that can be extracted from certain red seaweeds. They comprise a broad range of structures and can be divided into two families: the κ-family, defined by the presence of a C4-sulphate group on the β-D-unit, and the λ-family, characterized by the sulphate on C2 and constituted by all the variations of the λ-structures [83].

Investigations have detected antiviral activity in a significant number of algae from various marine environments such as California, United Kingdom, the Mediterranean, India and Japan [84]. In the course of a screening study on the biological properties of natural marine products, diverse carrageenans isolated from the red seaweed *Gigartina skottsbergii* Setch. et Gard. have proved to be potent inhibitors of HSV-1 and –2 *in vitro* [85]. Mode of action studies are consistent with the carrageenans having a major effect on the attachment of virus to the cells. Adsorption of HSV-1 is primarily mediated by the envelope glycoprotein gC, which binds to heparan sulphate residues present on the proteoglycans on the surface of target cells. Antiherpetic activity was directly correlated to the amount of α-D-galactose 2,6-disulphate residues in the natural carrageenans. Carrageenans were also isolated from Chilean samples of *Stenogramme interrupta* (C. Ag.) Mont., a red seaweed from California [86].

In the course of screening for antiviral properties in sulphated PS isolated from marine algae of the South American coast, Carlucci *et al.* [87] reported the antiviral activity of mannans and xylogalactans isolated from the red seaweed *Nothogenia fastigiata* Turner (Lam.), and a sulphated galactan from *Pterocladia capillacea* (Gmelim) Barnet & Thuset. HSV-1 and –2 were the most sensitive viruses of these compounds due to an inhibition of virus binding. Structural analysis of

two xylomannans extracted from *Nothogenia fastigiata* was carried out [88,89]. The results are consistent with the general pattern previously reported for other xylomannans of the same system, $\alpha(1 \rightarrow 3)$-linked D-mannans 2- and 6-sulphated and with single stubs of $\beta(1 \rightarrow 2)$-linked D-xylose, but one of the new samples contains a significant amount of 2,6-disulphated units. The presence of sulphate groups in the molecule was essential to the antiviral properties of these PS [90].

The antiviral activity of sulphated PS is known to increase with molecular weight or the degree of sulphation. However, sulphated PS are generally endowed with anticoagulant properties that may hamper their usefulness as antiviral drugs. Duarte *et al.* [91] reported the inhibitory effect of sulphated galactans from the marine alga *Bostrychia montagnei* Harvey. The results of the activated partial thromboplastin time assay and the thrombin time assay indicated that three natural sulphated PS have very low anticoagulant activity, confirming that there is no relation between the antiviral and anticoagulant properties.

Anti-HSV sulphated PS have also been isolated from other seaweeds such as rhamnan sulphate from *Monostroma latissimum* Wittrock [92], and calcium spirulan from the blue-green alga *Spirulina platensis* [62]. Lee *et al.* [93] initiated studies into the structure-activity relationships of calcium spirulan. Calcium ion binding with the anionic part of the molecule was replaced with various metal cations. Replacement of the calcium ion with sodium and potassium ions maintained anti-HSV-1 activity while divalent and trivalent cations reduced the activity. The cell wall sulphated PS of the red microalga *Porphyridium* spp. Honey exhibited impressive antiviral activity against HSV-1 and –2 both *in vitro* (cell culture) and *in vivo* (rats and rabbits) [94]. It seems that this PS is able to inhibit viral infection by preventing adsorption of the virus into the host cells and/or by inhibiting the production of new viral particles inside the host cells [95]. Novel fucan sulphates with anti-HSV activity were also isolated from the hot water extract of an edible brown alga *Sargassum horneri* [96] and from the brown seaweed *Leathesia difformis* (Linnaeus) Areschong [97].

Other natural sources of anti-HSV PS include fungi, e.g., various protein bound PS isolated from *Ganoderma lucidum*, a basidiomycetous fungus used to treat human diseases in oriental folk medicine [98]. The possible mode of antiviral activity of these PS seems to be related to their binding with HSV-specific glycoproteins responsible for attachment and

penetration, impeding the complex interactions of viruses with cell plasma membranes [99-101]. Furthermore, different semisynthetic PS were evaluated for their inhibitory effect on *in vitro* replication of HSV-1 and –2 [102-104]. Some neutral and negatively charged carbohydrates were able to inhibit viral infection by interfering mainly with the adsorption process.

The *in vitro* and *in vivo* effects of sulphated PS were also investigated against the pseudorabies virus (Suid HSV-1), the most notable of which is porcine HSV which has a well-documented history of epizootic infections, especially in Europe [105]. *In vitro* experiments revealed that sulphated PS significantly reduced the number of lytic plaques, but *in vivo* only heparin protected mice against the pseudorabies virus.

CYTOMEGALOVIRUS

In terms of their biological and pathogenetic properties, the herpes viruses fall naturally into several subfamily groupings, including cytomegalovirus (CMV), although detailed classification is at present premature. Nevertheless the CMV clearly constitute a group of their own with internal consistency. Human CMV is, together with HSV-1, one of the agents responsible for opportunistic infections in HIV-infected people.

As can be seen in the present review, sulphated PS show antiviral activitiy against enveloped viruses *in vitro*. It has been suggested that these antiviral mechanisms depend on their polyanionic properties, which interact with the positively charged amino acid sequence of viral envelope glycoproteins. Natural sulphated PS can act as potent inhibitors of CMV. The cell wall mucilages of seaweeds are known to be rich in sulphated PS with potential anti-CMV activity. Recently, Blinkova *et al.*[106] reviewed information on *Spirulina platensis*, a blue-green alga with diverse biological activity. Preparations obtained from this alga have been found to be active against several enveloped viruses, including CMV. It was revealed that calcium spirulan, a sulphated PS isolated from *Spirulina platensis*, selectively inhibited the penetration of the virus into host cells [62]. Retention of molecular conformation by chelation of calcium ion with sulphate groups may be indispensable to its antiviral effect. The antiviral activity of a polysaccharidic fraction obtained from another red seaweed, *Nothogenia fastigiata*, was also reported [107]. Its mode of

action against CMV could be ascribed to an inhibitory effect on virus adsorption.

Other natural sources of sulphated PS include brown algae. A sulphated polysaccharide with potent anti-CMV activity was isolated from the brown alga *Sargassum horneri* [68]. Time-of-addition experiments suggested that it inhibited not only the initial stages of viral infection, such as attachment to and penetration into host cells, but also later replication stages after virus penetration. Fractions of fucoidan, another polysaccharide isolated from the brown seaweed *Leathesia difformis* were also found to be selective antiviral agents against human CMV [97]. These compounds offer great promise as candidate drugs for the chemotherapy of CMV infections, and novel sulphated PS are being chemically prepared [108].

PS from terrestrial plants have also been reported as anti-CMV agents. For example, PS from three plant species, *Astragalus brachycentrus* D. C., *Astragalus echidnaeformis* Sirjaev and *Sterculia urens* Roxb., which are devoid of *in vitro* antiviral activity, were evaluated in mouse models of murine CMV infections [109]. Treatment with the compounds needed to be started one day prior to virus inoculation for maximum protective benefit. Treatments starting after virus inoculation were ineffective. The mannose-specific plant PS from the orchid species *Cymbidium hybrid* Cym., *Epipactis helleborine* (L.) Crantz. and *Listera ovata* (L.) R.Br. Svenska are potent and selective inhibitors of human CMV *in vitro* [110]. They presumably interact at the level of virion fusion with the target cell.

PAPILLOMAVIRUSES

Papillomaviruses are members of the Papopavirus family and are distinguished from other members by virtue of their association with a variety of warts in different parts of the body. Some types have been implicated in genital warts and cervical carcinomas, while others seem to be associated with other distinctive warts elsewhere. No generally effective control is available, although potentially dangerous lesions can be removed by cryosurgery or laser treatment. However, some medicinal plant preparations have been reported to be beneficial; conceivably these may work by promoting healing or stimulating immune responses, rather than directly inhibiting the virus.

Natural high-molecular weight sulphated or sulphonated PS, such as cellulose sulphate and dextran sulphate, may be useful non-toxic microbicidal compounds that are active against a variety of sexually-transmitted disease agents, including bovine papillomavirus type 1 and human papilloma virus type 11 and type 40 [111].

HEPATITIS VIRUSES

Hepatitis B virus (HBV) is widespread throughout human populations, specially in Asia and Africa, and it has been estimated that over 200 million carriers exist, some of whom are eventually expected to develop liver carcinoma or cirrhosis. HBV shows a strict tropism for liver hepatocytes in which it displays a protected replication with resultant foci of liver necrosis. The virus is a member of the Hepadnaviridae, along with several other species, and it replicates by a mechanism which appears to be unique to this family. In contrast, hepatitis A virus is a picornavirus and the hepatitis D agent appears to be a viroid-like RNA enclosed within a hepatitis B capsid, and consequently depends upon its association with the HBV for its spread and survival. Control may be effected by passive immunization (with hyperimmune globulin) or by various types of vaccines which are currently being developed and improved. Specific chemotherapy has not been consistently successful, but in some countries (e.g., India and China), plant extracts have provided some success.

Among natural antiviral agents of carbohydrate nature, sulphated PS such as λ and κ carrageenans showed a potent inhibitory effect on the replication of HBV in the human hepatoma cell line PLC/PRF/5 [112]. The two types of carrageenans resulted in concentration-dependent reduction of HBV-antigen expression and HBV infectivity, emerging as promising candidates for chemotherapy of acute hepatitis.

ROTAVIRUSES

Human rotavirus (HRV), a member of the Reoviridae, is a non-enveloped virus which is the major etiologic agent of severe dehydrating gastroenteritis in children worldwide. For the treatment of rotavirus gastroenteritis, intravenous fluid administration has been used successfully for dehydration from diarrhoea. However, in the case of

severe inpatients and immunocompromised hosts who are suffering from prolonged diarrhoea and fever, virus-specific treatment will be necessary if possible. Several compounds, biomaterials and plant extracts have been found to be inhibitory for HRV of some species *in vitro*. For example, extracts of *Stevia rebaudiana* Bertoni, a family member of Chrysanthemum originating from Paraguay in South America, inhibited the replication of all four serotypes of HRV *in vitro* [113]. Binding assays with radiolabeled purified viruses indicated that the inhibitory mechanism of this plant extracts is the blockage of virus binding. The inhibitory components of *Stevia rebaudiana* were found to be heterogeneous anionic PS with different ion charges.

INFLUENZA VIRUSES

Influenza continues to have a significant impact on public health. Annually, 20,000 deaths and 100,000 hospitalizations are attributed to "flu" in the United States alone. Airborne transmission, facile viral mutation, vaccine shortages, and actual and perceived side effects and limitations of both vaccines and prophylactic drugs contribute to the drive for new therapies and preventive medicines for influenza. Research during the last fifteen years has elucidated many of the mechanisms by which the influenza virus invades, captures and mobilizes the replication capabilities of a host cell, providing new targets in the search for antiviral treatments. For the past decades, besides a variety of synthetic antiviral drugs with different molecular targets, a large number of phytochemicals have been recognized to control infections caused by the influenza virus. Serkedjieva *et al.* [114] reported the antiviral activity from a lyophilized infusion from flowers of *Sambucus nigra* L., aerial parts of *Hypericum perforatum* L. and roots of *Saponaria officinalis* L. The preparations contain PS which could be responsible for their antiviral properties.

Reports on the anti-influenza virus activity of natural PS are recorded in the literature. From a pine cone extract of *Pinus parviflora* Sieb. et Zucc., an acidic polysaccharide was isolated [115,116]. This compound showed significant inhibition of both the viral protein synthesis in infected cells and virion-associated RNA-dependent RNA polymerase activity. In animal models *in vivo*, most mice inoculated intranasally or intracerebrally with lethal doses of the influenza virus A/WSN/33 died within 12 days. However, the infectivity of the virus that had been

preincubated with a polysaccharide prepared from cones of *Pinus parviflora* was significantly reduced [117]. In 1991, Sakagami *et al.*[118] reviewed the potential antiviral efficacy of natural polysaccharidic-related materials isolated from the pine cone extracts.

Sulphated PS, potent antiviral agents, were also evaluated *in vitro* as inhibitors of influenza virus replication [119]. The fact that the sulphated PS are inhibitory to some myxoviruses and retroviruses but not to others seems to depend on the composition of the amino acid sequences of the viral envelope glycoproteins that are involved in virus-cell binding and fusion [120].

As can be seen in the present review, other natural sources of antiviral sulphated PS include the marine environment. Sulphated and non-sulphated PS with anti-influenza activity have been isolated from marine sources such as the green marine alga *Ulva lactuca* L. [121], the Japanese sea alga *Crenomytilus grayanus* Dunkei. [122,123], the blue-green alga *Spirulina platensis* [106], the red seaweed *Nothogenia fastigiata* [107] and the marine microalga *Cochlodinium polykrikoides* [67]. Novel marine PS which have proved to be potent and selective inhibitors of the influenza virus *in vitro* are being chemically synthesized [124,125].

MEASLES VIRUS

The measles virus (MV) is a paramyxovirus which has been associated with several chronic diseases. Since the advent of mass immunization campaigns, the incidence of measles has decreased dramatically in many developed countries. This is not the case elsewhere, however, where the virus still takes its toll in large numbers, especially in malnourished or immunocompromised individuals. Secondary bacterial infections are prevalent in these populations. Although most infections are relatively short-lived and innocuous in healthy individuals, encephalitis can develop in approximately 0.1% of cases; hence vaccination is desirable. Following respiratory infection, the virus readily invades local lymphoid tissues and gains access to the blood, from where it disseminates widely in the body. There has not been much demand for chemotherapy; vaccination seems to be the choice. No really effective antiviral has been evaluated, although some plant compounds have been shown to inhibit MV replication effectively, such as PS from the blue-green alga *Spirulina platensis* [62].

RABIES VIRUS

Rabies has long been recognized as a scourge of livestock with occasional and often fatal intrusions into humans and their pets. Initially the virus gains access to muscle, sensory organs or skin, and there replicates in local unmyelinated (sensory) nerve fibers. Many layers of epithelial cells are susceptible to the virus but the principal target is the unprotected neuron. The brain is where most damage is done, resulting in the familiar psychomotor disturbances. In addition, the virus spreads into salivary glands (by axoplasmic flow again), from where it is secreted from the asinar mucous cells and transmitted to the victim. The objective of much research on the rabies virus has been the development of better vaccines and therapeutic measures, some of them from natural sources. For example, different natural polymeric carbohydrates inhibited rabies virus infection in chicken-embryo-related cells by interfering with the virus adsorption process [126]. The charge density and the polymeric backbone of the molecules seem to play a role in influencing the antiviral properties, whereas other features such as the sugar moieties do not appear to be relevant.

TOGAVIRUS

Rubella is the sole member of the Rudivirus genus in the Togavirus family. The virus is transmitted by the respiratory route, and multiplies in upper respiratory tissues from where it gains access to the blood, accompanied sometimes by a characteristic rash and other symptoms. The infection may be more severe in pregnant women. Vaccination programmes are in place in many countries, and the belief is that the virus will eventually be brought under control. Although there seems to be no apparent role or need for specific antiviral therapy, reports on the anti-rubella activity of natural products and extracts have been reported [57]. For example, the natural carbohydrate scleroglucan, three sulphate derivatives and dextran sulphate were investigated for their inhibitory effect on rubella virus infection on Vero cells [127]. The results indicated that PS blocked a step in virus replication subsequent to virus attachment, such as internalisation and/or uncoating.

BUNYAVIRUS

Sandfly fever Sicilian virus (SFSV) is a Phlebovirus, a member of the Bunyaviridae family, which cause acute, incapaciting but self-limiting diseases in man, and which have had a major impact in Europe, the Middle East and Africa. SFSV is a virus closely related to other viruses which are considerably more pathogenic both in humans and animals, such as the African Swine fever virus (ASFV) and the Crimean-Congo haemorrhagic fever virus (CCHFV).

The ASFV is a highly contagious bunyavirus infection of pigs which is clinically indistinguishable from the unrelated hog cholera virus. Since 1957, epidemics have been recorded intermittently in several European countries bordering the Mediterranean, in the Caribbean, and in Brazil, and more recently in other European countries and parts of Africa. Although pigs appear to be the only species infected naturally, the virus can be adapted to grow in a variety of other cell types and experimentally infects goats and rabbits. In view of this, it seems worthwhile to consider the possibility of its natural persistence in animals other than swine and man. Transmission of ASFV usually occurs through the respiratory route. Following inhalation, the virus invades and attacks local lymph nodes and the endothelium of blood vessels, with the result that high titers of virus circulate in the blood and lymph, and eventually in various secretory fluids. Attempts have been made to produce vaccines, but without much success. In any case, the virus mutates frequently in the wild and cross-protection of animals is not complete. Since the virion contains several enzyme activities, and the DNA can probably code for more in the infected cell, the possibility of specific chemotherapy, along the lines of antiherpes chemicals, should be profitable.

Several studies have been undertaken to develop drugs which could be used for treatment of fever infections caused by viruses belonging to the Bunyaviridae family, including natural PS [128,129]. Antiviral activity was estimated by the reduction of the cytophatic effect of SFSV on infected Vero cells. Several PS, such as carrageenans, fucoidan, dextran sulphate and pentosan polysulphate caused a concentration-dependent reduction in the virus yield. Fabregas *et al.* [130] screened several extracts from marine microalgae for *in vitro* inhibition of the replication of ASFV. That this inhibition could be due to sulphated PS was suggested because the same pattern of viral inhibition was obtained by using exocellular

extracts from microalgae enriched in these compounds and/or dextran sulphate of high molecular weight.

Other natural PS should be considered for the potential treatment of significant human infections caused by bunyaviruses. The mouse model of Punta Toro virus, a Phlebovirus member of the Bunyaviridae family, is a model for studying the treatment of Rift Valley fever infection. Tragacanthin PS from *Astragalus brachycentrus* and *Astragalus echidnaeformis* plants caused reduction in mortality, liver infection scores, liver and spleen virus titers, and serum transaminases in treated mice [131].

POLIOVIRUSES

Poliomyelitis is not the scourge that it once was, thanks to improved hygienic practices which have effectively blocked the spread of wild-type polioviruses, especially in developed countries, and thanks to the widespread use of vaccines. The viruses, like other enteroviruses, are transmitted by the fecal-oral route; following ingestion, they replicate in various cell types in the pharynx and small intestine (they are quite stable to stomach acids) and gain access to the circulation. From there, they disseminate and occasionally gain access to the central nervous system, where they may produce the characteristic lesions leading to poliomyelitis.

Control of virus spread accompanied by vaccination continues to provide the best means of alleviating poliomyelitis. The use of antivirals seems to have less application than for many other viral infections, although the poliovirus continues to serve as a useful laboratory model for antiviral screening. The plant kingdom and seaweeds may be a source of potentially useful and interesting antiviral compounds against polioviruses. For example, anti-poliovirus activity was detected in a polysaccharidic-rich fraction from extracts of several Korean seaweeds [132], and from the leaves of the Meliaceae tree *Trichilia glabra* [81].

OTHER VIRUSES

Other compounds of carbohydrate nature with antiviral activity against other viruses have been isolated from natural sources. Several compounds

belonging to different classes of sulphated PS were evaluated for their inhibitory effects on the replication of the arenavirus Junin and Tacaribe in Vero cells [133,107]. Very potent and selective inhibitors were the sulphated PS dextran sulphate, λ-carrageenan, fucoidan, heparin and pentosan polysulphate. Examples also include aloe polymannose, a high mannose purified from the *Aloe barbadensis* Miller plant, which showed activity in enhancing antibody titers against coxsackievirus B3-induced myocarditis in murine models of the disease [134], and fungal PS which are active *in vivo* in Sendai virus infection [135].

Although relatively little work has been done on natural antivirals against plant viruses, several reports concerning antiviral activity against plant virus infection have been recorded; for example, yeast mannans with antiviral activity against the tobacco mosaic virus infection in tobacco plants [136], and lichenan PS from Iceland moss which exhibited antiviral activity against the potato viruses [8].

ABBREVIATIONS

PS = Polysaccharides
AIDS = Acquired Immunodeficiency syndrome
HIV = Human immunodeficiency virus
SIV = Simian immunodeficiency virus
HIV-RT = HIV-reverses transcriptases
HSV = Herpes Simplex Virus
CMV = Cytomegalovirus
HBV = Hepatitis B virus
HRV = Human rotavirus
MV = Measles virus
SFSV = Sandfly fever Sicilian virus
ASFV = African Swine fever virus
CCHFV = Crimean-Congo haemorrhagic fever virus

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