Review Article

Innovative Scientific Concept of Topical Virus Glycoprotein Inhibitors Incorporated in Hyperosmotic Glycerol Revolutionizes Future Prospects in the Treatment of Viral and Bacterial Throat Infections

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ABSTRACT

Pharyngitis is primarily a viral infection of the respiratory tract, followed by secondary bacterial invasion due to weakening of local defenses. Influenza A and rhinoviruses are principally involved, the influenza virus having much higher pathogenicity, however, than rhinoviruses which usually cause the common cough and cold without severe damage to the respiratory mucosa. Following initial infection, the virus enters the cells only for multiplication and almost all virulent virus particles subsequently produced are shed onto the throat surface. From the throat surface, the virions infect new healthy cells, damaging the throat mucosa, and creating a favorable ground for secondary bacterial colonization which is the cause of almost all symptoms of throat infection (sore throat, strep throat). The virus' complex structure, its constant mutation, the variety of its surface glycoproteins, as well as the role of topical proteases helping virus entry and virus – bacteria symbiosis must all be taken into account in designing an effective treatment, acting on multiple parameters. As most viruses and all bacteria are present on the throat's outer lining, treatment should be designed to act topically on the surface of the pharynx, which also minimizes side effects. Until 2012, no topical antiviral drugs were available and almost all treatment strategies were directed to relieve only the symptomatic manifestations of throat infections. Anti-influenza vaccination is still considered the best preventive measure, while the use of intracellular virus inhibitors is strictly limited to severe cases as they were not found to be very effective once throat infection is established. The recent development of non-specific topical virus glycoprotein inhibitors, incorporated in a filmogen glycerol solution for an increased duration of action, represents a breakthrough yet relatively simple scientific approach for the treatment of viral throat infections accompanied by secondary bacterial infection. In this review, we analyze the whole process of viral throat infection, virus – bacteria interactions on the throat surface, currently available treatments and their drawbacks, and this innovative therapeutic approach consisting in virus glycoprotein inhibitors in an osmotic solution, destined to totally change the future treatment of throat infections.

Keywords: antiviral, influenza virus, osmotic, pharyngitis, tannins, throat.

INTRODUCTION

The common cold and throat infections are the most frequent illnesses in the world. Characteristic symptoms include cough, cold, throat pain, sudden onset of fever, weakness, runny nose, and headache, occasionally followed by rhinitis and rhinosinusitis. Most people recover within one to two weeks, when the body's defense mechanisms are activated and neutralize the causative pathogen, but in weakened populations the infection may spread and cause complications, especially with infections by influenza A and B viruses. [1]

Depending on the epidemic's severity, nearly 20% of the world population suffers each year from common cold-associated throat infection, costing 100s of billion Euros in healthcare and lost productivity, whereas the influenza virus affects about 5 to 15% of the population in Europe [2] and the US, generating economic losses between US$ 70 & 170 billion each year (WHO estimation). [3] Every year, influenza epidemics are thought to result in 3 to 5 million severe cases in Europe alone, and between 250 000 and 500 000 associated deaths worldwide, most deaths in industrialized countries occurring among the elderly over 65 years of age. [2]

Throat infections are chiefly of viral etiology, with secondary bacterial infection ensuing, as both pathogens often act symbiotically. Although viral pathogenesis is a prerequisite for subsequent infections, viruses and bacteria may be present in the naso-pharynx without causing any respiratory symptoms. The upper respiratory tract hosts a complex microbial community which is assumed to be constantly

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subject to synergistic and competitive interspecies interactions, but disturbances in the equilibrium of bacterial or viral populations may lead to overgrowth and invasion. [4]

In the majority of throat infections, only a few virus particles first come into contact with the pharyngeal mucosa, then start multiplying and liberating a huge amount of active free virus particles on the throat surface. When newly liberated virions attack healthy cells, the dead and dying cells and resulting minute mucosal damage create an environment favorable to opportunistic bacterial growth. Recent findings suggest that viruses and bacteria do not fight; on the contrary they may even cooperate to spread the infection. Therefore, suppressing the initial viral infection is key to preventing serious throat illness. If the infection is already established, associating a topical antiviral agent to a topical antiseptic becomes necessary to stop further infection and rid the throat of contaminants. Many antivirals, antibacterials, antiseptics and common cold treatments are available, but none of these has the combined antiviral and antiseptic properties essential to treat throat infection, whereas current antiviral drug development is still limited to intracellular virus growth inhibitors. [5] The complexity of throat infection, necessity of a multi-target approach, impossibility of associating different molecules within a single drug and patenting a combination product, have totally hampered pharmaceutical R&D for a curative pharyngitis drug. [6] This review focuses on the recent development of topical virus glycoprotein inhibitors incorporated in glycerol as a topical antiviral and antiseptic throat treatment free of side effect, a concept which may radically change the usage of existing antiviral drugs as well as future antiviral research. We shall briefly examine the events inherent to viral throat infection, the symbiotic mechanisms of virus – bacteria interactions which cause and maintain throat infection, the available antiviral and antibacterial drugs, and the recently launched antiseptic-antiviral treatments destined to revolutionize the future of antiviral research targeting throat infections.

Main causes of throat infection

The predominant pathogens in upper respiratory tract infections (URT) are viruses of the *Orthomyxoviridae* (influenza) and *Paramyxoviridae* (including the parainfluenza viruses (PIVs), human respiratory syncytial virus (RSV), and human metapneumovirus (hMPV) families, whose members are enveloped viruses. Nasopharyngitis may also involve other viruses, with milder frequency and pathogenicity. These viruses include: the omnipresent common cough rhinoviruses (single-stranded, non-enveloped) whose multiplicity of serotypes (over 100) seriously handicaps vaccine development; enteroviruses (coxsackieviruses and numbered enteroviruses); and enveloped viruses (*Coronaviridae* family), containing positive-sense single-stranded RNA (ssRNA), among which the human coronavirus (HCoV) 229E, HCoV OC43, the severe acute respiratory syndrome-associated CoV (SARS-CoV), and the HCoV NL63 and HCoV HKU1 viruses, are known human pathogens. [7] Other DNA viruses involved in human upper or lower respiratory tract infections comprise non-enveloped double-stranded DNA (dsDNA) viruses (*Adenoviridae*), and the ssDNA human bocavirus (HBoV) (*Parvoviridae*). This review concentrates particularly on viruses from the *Orthomyxoviridae* family (containing influenza A, B, and C groups) since they are the prevalent infective agent of pharyngitis, type A and B viruses causing epidemic flu illness, while the less common type C causes isolated mild disease, mostly in children. [8] The genome of Orthomyxoviruses is composed of minus-strand RNA, containing 6 to 8 segments. Influenza A and B species comprise three transcriptases (PB1, PB2, and PA); two surface glycoproteins, hemagglutunin (H or HA) and neuramidase (N and NA); two matrix proteins (M1 and M2); and one nucleocapsid protein (NP). Sequence and antigenic analysis allowed differentiating eighteen H (H1-H18) and eleven N (N1-N11) subtypes in animal and avian strains, among which only H1, H2 and H3 and N1 and N2 are known to engender widespread human epidemics. [9-10] However, mutations in progeny genetic structures often occur during influenza virus replication and the minor genetic changes (known as antigenic drift) demand yearly influenza vaccines reformulation. [11-12]

Initial virus attack

In human beings, influenza typically causes sore throat, and sometimes pneumonia. Virus transmission is either airborne, as cough and sneezes produce aerosols which carry the virus, or through nasal secretions or contact with contaminated surfaces. Viral infections' mode of progression differs completely between topical and systemic infections. In an external topical infection such as influenza, a few virus particles initially come into contact with throat mucosal cells, with practically no clinical signs at this stage. After initial contamination, the virus multiplies inside the cells and millions of virions are then liberated topically, and in turn infect new cells and eventually create visible lesions. [13]

Role of proteases

Proteases, also known as proteinases or proteolytic enzymes, are a large group of enzymes found inside or outside the cells, particularly in the vicinity of damaged tissues and chronic wounds. Involved in the splitting of protein molecules (catabolism), they have an essential role in creating an environment conducive to tissue repair, by facilitating removal of proteinous debris generated during tissue breakdown and interfering with the healing process. Proteases are divided into four major groups according to their mode of action: metalloproteinases or Matrix-Metallo-Proteins (MMPs), serine proteinases, cysteine (thiol) proteinases, and aspartic proteinases. Their exact number is not yet known, however, as new proteases are being discovered regularly. [14]

They are also implicated in virus-related processes. Virus entry is intimately dependent on membrane fusion, whose activating factor is the host cell's HA (0) protease. [15] At least seven different trypsin-type processing proteases, including tryptase Clara and tryptase TL2, have been identified for HA (0) processing but there are probably many others not yet identified. [16-18] In addition to the proteases present on the infected throat surface, intracellular virus multiplication also encodes up to 11 proteins and this coding capacity demands that the virus use the host cellular machinery for many aspects of its life cycle, [19] including the help of different intracellular proteases. Thus, the influenza virus uses some specific proteases or enzymes present on the surface of the respiratory tract to penetrate and infect throat cells. To restrict viral infection, our body defense mechanisms liberate anti-proteases, called secretory leukoproteases in the URT and pulmonary surfactants in the lower respiratory tract, to reduce the amount of proteases available to assist viral entry. But when protease activity

predominates over the activities of inhibitory compounds, viral infection cannot be stopped. [20] Body defense mechanisms are activated to produce antibodies and to stop virus replication but this normally takes 5-10 days. This is the reason why protease inhibitors may be pivotal and are being considered as potential future therapeutic agents for the treatment of influenza. [17, 21]

Virus surface glycoproteins

All enveloped viruses possess specific proteins, called glycoproteins (Gps), on their surface. These Gps code for virus antigenicity. As throat infection is caused chiefly by the influenza virus, we will restrict our Gp description to this virus. A and B influenza species are difficult to differentiate even by microscopic examination, both appearing spherical or filamentous in shape, their size ranging from 100 nm in diameter for spherical forms to frequently over 300 nm in length for filamentous forms. Virions of the A species have Gp spikes, roughly 4 HA for 1 NA, jutting out from a host cell–derived lipid membrane, [22] traversed by matrix (M2) ion channels, with approximately one M2 channel per 10^1-10^2 HA molecules. The envelope and its three integral membrane proteins HA, NA, and M2 enclose the matrix of M1 protein wrapping the central virion core. The nuclear export protein (NEP, aka nonstructural protein 2, NS2) and the ribonucleoprotein (RNP) complex, which consists of the viral RNA segments coated with nucleoprotein (NP) and the heterotrimeric RNA-dependent RNA polymerase, composed of two "polymerase basic" and one "polymerase acidic" subunits (PB1, PB2, and PA) are inner components of the matrix. The influenza B virion structure is comparable (its envelope possessing four proteins, though: HA, NA, plus NB and BM2), whereas that of influenza C virions is different: on infected cells, they can organize in long strings on the order of 500μm. However, influenza C virions are compositionally similar: the matrix protein M1, enclosing the RNP and polymerase complex core, is covered by a lipid envelope with a sole major surface Gp, the hemagglutinin-esterase-fusion (HEF) protein, whose function is equivalent to that of HA and NA in A and B types, and one minor envelope protein, CM2. [23-24] The HA projecting from the influenza virus identify and bind the N-acetylneuraminic or sialic acid moiety on the host cell surface, favoring α-2, 3- or α-2, 6-linkages. The NA not only assists virus attachment to cells but could also enhance virus infectivity by facilitating access into the respiratory epithelium and epithelial cells through mucin disintegration [25] and cleaving of sialic acid, which may regulate HA binding to the host cell surface. Deleting NA from the influenza A virus' genetic sequence dramatically curbs initiation of infection as nascent virions then aggregate, incapable of dispersing through a cell monolayer. [26-27] Since full infection is obtained through HA and NA cooperation, a topical antiviral drug should target both surface Gps for efficient virus inhibition. [28] The virus penetrates the cell by endocytosis mediated by HA (or HEF in influenza C virus) – sialic acid binding. The acidic environment inside the endosomal compartment is necessary to virus uncoating, causing the HA to change shape and bare a fusion peptide to mediate the fusion between virus envelope and endosomal membrane: through this minute aperture, the virus' genome penetrates into the host cell cytoplasm [29] and the released RNPs are then imported into the host cell nucleus through viral proteins' nuclear localization signals (NLSs). [30] The nucleus is the synthesis

site of all influenza RNA (messenger-RNA (mRNA) as template for host-cell translation of viral proteins, and negative strand viral RNA segments as components of progeny genomes). Membrane-bound ribosomes decode viral mRNA to synthesize HA, NA, and M2 proteins into the endoplasmic reticulum, to be conveyed to the Golgi body for post-translational modification. Apical sorting motifs on HA, NA, and M2 transmembrane envelope proteins are recognized by the trans-Golgi network and determine their route towards the site of virion assembly (including the vRNP core) and morphogenesis, budding and release (likely initiated by M1 matrix protein): the plasma membrane. After budding is complete, HA-mediated binding of virions to host cell surface sialic acid continues until NA's sialidase activity releases the virion progeny. [25, 30] Host antibodies to the NA, or antiviral neuraminidase-inhibiting drugs, stop infected cells from expulsing the virus and inhibit viral replication. Shedding of fresh virus particles onto the infected surface after initial contamination perpetuates new host cell infection, and when sufficient cellular damage is done, microscopical lesions appear on the surface of the pharynx where opportunistic bacteria, normally harbored there, start proliferating and cause secondary bacterial infection, much more detrimental to the throat surface than the damage caused through virus replication.

Secondary bacterial infection

A complex ecosystem of commensals and opportunistic pathogens (pathobionts), including *Streptococcus pneumoniae* (pneumococcus), *Haemophilus influenzae*, *Moraxella catarrhalis* and *Staphylococcus aureus*, populates the human URT. [31] Most of these bacterial species inhabit the nasopharyngeal mucosa in healthy individuals but their infectivity is neutralized by the body's defenses and a healthy mucosal barrier. For throat infection to begin, bacterial proliferation and colonization of URT mucosa must take place, which occurs much more easily in children. [32] In some cases, different bacterial species strive against each other while in other cases they collaborate for mutual benefits. [33-34]

Colonizing tactics include production of noxious hydrogen peroxidase (H_2O_2) by certain bacteria that are virtually immune to it (such as the highly H_2O_2-tolerant *S. pneumonia*), in concentrations lethal even for bacteria able to produce the H_2O_2-neutralizing enzyme catalase, such as *S. aureus* [35] and *H. influenzae*. [36] Another strategy consists in preventing a competing microorganism from adhering to the host epithelial surface. For example, *pneumococcus*-expressed NA clips sialic acid from the lipooligosaccharides of some *H. influenzae* strains' outer membranes, preventing virus adhesion to nasopharyngeal cells and subsequent colonization. [37] Bacterial adherence to host cell receptors may also be mediated by phosphorylcholine, a cell-surface molecule expressed by both *S. pneumoniae* [38] and *H. influenzae*, [39] but essential only for the survival of *pneumococci*. *H. influenza* can therefore produce phosphorylcholine, trigger host immunological reaction to produce antibiodies, and neutralize *S. pneumoniae*. [39-40] The host immune system is also involved in interspecies competition, by eliminating one species through complement- and neutrophil-mediated killing [41-42] or by helping the survival of other species through immune evasion. [43] Microbial interactions appear to involve a complex interplay between multiple host factors and bacterial

characteristics, with a significant impact on both the severity of microbial infection and the strategy to develop an effective antibacterial drug. [44] Therefore, depending upon the state of immunity of the host and the predominance of bacterial species on the throat surface, the type of bacteria colonizing the nasopharynx may vary, requiring a broad spectrum antimicrobial approach for throat infection relief.

Viral-Bacterial symbiosis
While throat infection is usually of viral origin, the clinical symptoms are predominantly imputable to secondary bacterial infection. Although mortality from influenza alone is possible, clinical severity of the condition increases dramatically when aggravated by bacterial surinfection. Strong interactions exist between respiratory tract pathogenic viruses and native bacteria, particularly between influenza virus and *S. pneumonia.* [45] Recent findings indicate that these two pathogens act symbiotically.

The key mechanisms by which viruses cooperate to enhance bacterial infection may include:

Bacterial adhesion to throat mucosa
Viruses sap the host epithelium defenses and render URT mucosal surfaces even more vulnerable to pathogen attachment and subsequent colonization. [46] Virus-aided bacterial attachment occurs not only in presence of simultaneous infection, but also up to a week after initial viral infection [47-48] or even after full recovery from the flu. [49]

Throat mucosa cell lyses
After initial attachment of a few virus particles, viruses grow inside a few throat mucosal cells, induce cell lysis, and eventually damage the epithelial layer, [50-51] exposing the basement membrane matrix. It was observed that *S. pneumonia,* [52] *S. aureus* and *M. catarrhalis* [53] bind to ECM proteins, suggesting that these species could take advantage of this denudation. Cellular damage also triggers the production of fibronectin which further heightens bacterial binding to the throat mucosa. [48] Mucosal damage results in loss of epithelial integrity and decreased inhibition of bacterial translocation, as illustrated by rhinovirus-induced paracellular migration of *H. influenza.* [54] Ciliated cells' mucociliary velocity and barrier functions may also be deteriorated by viruses. [51, 55]

Expression of defensin proteins
Inside host cells, the virus may cause changes in the expression of antimicrobial peptides, or defensins, [56] secreted in airway mucosa, and whose essential innate immunity role is to eradicate harmful bacteria. [56-57]

Topical inflammation
In epithelial cells, the inflammatory response prompted by viral infection induces the upregulation of adhesion molecules which act as receptors mediating the attachment of immune cells to virus-infected cells to fight and clear the infection, as illustrated by the intracellular adhesion molecule 1 (ICAM-1), outer membrane protein P5-homologous fimbriae (P5 fimbriae), carcinoembryonic adhesion molecule-1 (CEACAM-1), and platelet-activating factor receptor (PAFr) in different cell types upon infection with a virus such as RSV or PIV. [48, 58] However, some bacterial species also bind to some of these adhesion proteins on the surface of host cells. [48, 59-61] For example, upregulation of ICAM-1 instigated by rhinovirus for its own invasion will also be used by *H. influenza.* [48, 62] Viral infection also increases expression of natural PAFr-ligand

phosphorylcholine by certain strains of *S. pneumoniae* and *H. influenza*, which further facilitates their adhesion and invasion. [48, 60-61]

Neuraminidase production by influenza virus
Influenza viruses produce NA whose essential function of cleaving terminal sialic acid residues clears the path for bacteria to reach their receptors on the surface of the URT. [63-64]

Cellular mechanisms
Virus infection increases adhesion of neutrophils, monocytes, and other immune cells to virus-infected cells, resulting in pro-inflammatory immune response. It also increases susceptibility to bacterial superinfection by inducing impairment of neutrophil function, diminution of oxidative burst, [65-66] and intensified neutrophil apoptosis. [66-67] Some influenza virus strains may predispose to superinfection by *S. aureus* due to poor recruitment and activation of natural killer (NK) cells. [68] Viral infection may also modify biological functions of monocytes, resulting in lower CD receptors surface expression, [69] as well as of cytokines. [70] Thus, virus-induced interferon (IFN)-α and IFN-β prompt ineffective neutrophil responses due to a diminished production of neutrophil chemoattractants. [71] Moreover, IFN-γ decreases the activity of macrophages, [72] undermining the first line of bacteria removal. Tumor necrosis factor (TNF)-α production is also downregulated during viral infection, resulting in higher vulnerability to secondary bacterial infections. [68] This proves that secondary bacterial infection is a very common phenomenon underlying complex interactions between bacteria and viruses during viral throat infection. Therefore, to be effective, a treatment should not only possess antiviral but also strong antibacterial properties.

An ideal treatment approach
An effective treatment should be capable of neutralizing the free virus particles present on the surface of the throat, so as to stop the primary cause of infection. But taking into account the amount of virions on the infected surface, the role of virus entry-enhancing proteases and the extracellular location of the virus in conjunction with the microbial infection, a multi-level approach of inhibiting the virus infection, neutralizing virus entry-enhancing proteases as well as detaching and eliminating microbial contaminants from the throat surface is essential to stop and cure the infection. The treatment must be as rapid as possible, without side effects and without altering the normal functions of healthy cells.

Currently available antivirals
Antiviral drugs constitute one of the biggest research areas of the pharmaceutical industry. An ideal antiviral drug should inhibit virus replication when used at concentrations not detrimental to the host, should be non-toxic and non-irritant if applied topically. Viruses infecting the pharynx are mostly present on the epithelial surface of the throat although a small number of virus particles continue multiplying inside the cells. Infected cells are bound to die and their lyses keep on liberating new virions onto the throat's surface. Stopping new infection is therefore the 1st fundamental step for treating a viral throat infection.

Current treatment strategies include the use of vaccines, intracellular virus growth inhibitors such as amantadine, rimantadine, ribavirin, idoxuridine, trifluridine, vidarabine, acyclovir, ganciclovir, foscarnet, zidovudine, didanosine,

zalcitabine, stavudine, famciclovir and valaciclovir, and throat gargarisms, but their modes of action are not adapted for the treatment of throat infections. These drugs are mostly used orally to treat herpes virus, enterovirus, and, to a lesser extent, rhinovirus and severe influenza infections, mainly by inhibiting viral development at various stages of the virus' replication cycle.

Roughly, the virus replicative cycle can be divided into 10 stages: (1) adsorption, (2) penetration, (3) uncoating, (4) early transcription, (5) early translation, (6) replication of the viral genome, (7) late transcription, (8) late translation, (9) assembly, and (10) release of new virus particles. The first three replicative stages are specific to virus infection and do not occur in uninfected cells. Examples of virus replication steps controlled by virus-specified enzymes include transcription of positive-sense RNA to DNA (catalyzed by the reverse transcriptase associated with retroviruses), replication of DNA to DNA (catalyzed by the DNA polymerases of herpes viruses), and proteolytic cleavage of viral precursor proteins (catalyzed by the protease of human immunodeficiency virus), but these events occur inside the cells and require the drug to be present inside the body. For the treatment of throat infection, an antiviral drug present systemically and acting on the viral replication may not have any effect on the free virions attacking the throat surface from the outside. The mode of action of the commonly used antiviral drugs is as follows:

Amantadine and rimantadine
These two antiviral agents are administered orally and are suggested in severe cases of influenza A. They block the H^+ ion channel of the M2 protein membrane found on influenza A viruses. This inhibits the acidification of the interior of the virus and ultimately prevents the release of viral RNA. [73] When administered orally within 48 hours of the onset of symptoms, these drugs can reduce the severity of symptoms and lessen the duration of influenza A illness by approximately 24 hours. Despite their efficacy, these antivirals are not widely used because they have no effect on virions present on the throat surface, lack activity against influenza B viruses, carry significant risk of side effects for the central nervous system (especially amantadine), and can rapidly select for drug resistance mutations during treatment.

Acyclovirs
Related to cytarabine, idoxuridine, trifluridine and vidarabine, acyclovirs are nucleoside analogue antiviral drugs. Like the earlier antivirals, acyclovir only shows activity against some members of the herpes group among the DNA viruses. Despite evidence of efficacy in ocular herpetic keratitis, as well as initial and primary genital herpes infection, acyclovir offers virtually no clinical benefit in throat infection. Topical ointment proved moderately helpful in treating recurrent genital herpes by shortening its course by a couple of days. Oral and intravenous acyclovir was beneficial in initial genital herpes infections as it abbreviated recurrent outbreaks by 1 to 2 days, but had no effect on pain and other symptoms. In non-immunocompromised patients with recurrent herpes simplex labialis, little clinical amelioration was derived from topical acyclovir ointment, even with therapy initiation in prodromal phase, while topical acyclovir cream produced modest yet significant improvements in the clinical but not the symptomological course of the disease. [74] Despite some aspects of the drug's use in severe herpes infection, acyclovir-containing drugs have no topical effect on virus growth and have multiple side effects when given orally.

Neuraminidase inhibitors
NA is a surface Gp common to both types of influenza. Its enzymatic activities are essential for the release of virions from infected cells and prevention of virus aggregation at the host cell surface. [75] NA inhibitors, such as oseltamivir and zanamivir, have no effect on virus present on the throat surface but can used orally or by inhalation for the treatment of uncomplicated acute influenza A and B infections if administered within 48h of onset of symptoms as otherwise these drugs have no beneficial effects, [76-77] and may cause adverse effects (such as transitory nausea with oseltamivir). [78] Because early administration of these drugs is essential, yet they have no efficacy on free virus particles present on the throat surface, have minor but multiple side effects [79] and are known to develop virus resistance to neuraminidase inhibitors, [80] they are limited to severe cases.

New intracellular antivirals
Many new antiviral drugs are under development but are almost all directed to stop intracellular virus growth and require oral administration. Because of the close interaction between virus replication and normal cellular metabolism, it is very difficult to interrupt the virus replicative cycle without adversely affecting host cell metabolism. However, some of the events in the virus replicative cycle either do not occur in normal uninfected cells or are controlled by virus-specific enzymes that differ structurally and functionally from the corresponding host cell enzymes. Future antiviral drug research is directed to act on these events to avoid side effects. [5-6]

As indicated before, during respiratory infection, most of the virus is present on the infected surface, and only a topical antiviral drug may have the therapeutic potential to reduce the continuously perpetuated virus attack. However, such treatments may not be able to cure the infection if they do not neutralize virus entry-enhancing proteases and get rid of the microbial contaminants.

Vaccines
Vaccination is the most effective strategy to prevent, or lessen the severity of, influenza and subsequent throat infection. But, despite the influenza-associated morbidity and mortality, vaccines are used in barely 30% of the population. [81] Vaccine efficacy is contingent on the antigenic match between the strains in the epidemic and those contained in the vaccine, as well as on the recipient's age and immune status. Yearly vaccine reformulation comprises the two type A and one type B strains with the highest probability of circulating that season. Adequate antigenic correspondence allows curbing influenza infection or severity in roughly two thirds of the vaccinated population, helping protect them, if not from pharyngitis, from the more serious complications leading to hospitalization or even death. [82] New vaccines able to completely block the infection are needed to convince "at risk" populations to accept vaccination. Vaccination also presents the disadvantages of a lag period between inoculation and its effectiveness (approximate 3 weeks), absence of efficacy to neutralize free virions on the throat surface, necessity of medical personnel for injection, relatively high cost, and poor antigenicity profile in many cases. The use of trivalent, live, attenuated intranasal vaccine in the future is under assessment as such vaccines may offer the advantage of eliciting specific mucosal innate immunity

responses resulting in significantly higher protection against influenza than inactivated vaccines. However, initial tests show that although incidence of severe febrile illness and febrile URT infection was lessened by trivalent live attenuated vaccination compared to placebo groups, infection and fever still persisted. [83]

Antibiotics and anti-inflammatory drugs

Antiobiotics have no effect on viral growth but the use of antibiotics to avoid secondary bacterial complications is very prevalent, particularly in developed countries. *Streptococcus* and mainly group A β-hemolytic *streptococcus* (GABHS) are commonly found in throat infections. GABHS are very sensitive to penicillin V because the bacterium cannot manufacture β-lactamase. First-line drugs for bacterial pharyngitis therefore include penicillin, ampicillin or amoxicillin. [84] Erythromycin and first-generation cephalosporins (CG) represent reasonable alternative treatments, particularly in cases of non-life-threatening allergy to penicillin, failed response to penicillin or re-infection following penicillin therapy. [84-85] Since GABHS is a prevalent infective agent, fluoroquinolones, and sulfamethoxazole/trimethoprim showing poor activity against Gram-positive pathogens are not the best choice of antibiotics, whereas the more efficient amoxicillin-clavulanate, clarithromycin, azithromycin and second-generation cephalosporins are only third-line options as their broader spectrum heightens bacterial resistance potential. A 5-7 day course has proved clinically effective in reducing bacterial load, but mean duration of illness is shortened by less than 1 day, with no difference in time off from work, and antibiotics have generally limited effect on sore throat symptoms while presenting the risk of causing resistant bacteria strains to emerge. [86-87] Headache, pain, and fever are usually slightly less severe when patients having positive GABHS cultures are treated with antibiotics. Antibiotherapy would also help reduce the risk of pharyngitis being compounded by other local infections (e.g. otitis and sinusitis). However, these minor benefits must be weighed against antibiotics-induced side effects such as vomiting, diarrhea, abdominal pain, and rashes, as well as allergic reactions to penicillin. [88]

In addition to antibiotherapy, corticosteroids, including cortisone, hydrocortisone and prednisone, also constitute a common symptomatic relief treatment, chiefly to alleviate throat pain in participants with severe URT infection. [89] Corticosteroids mimic the effects of adrenal hormones that can reduce the signs and symptoms of inflammatory conditions but can also suppress the immune system, which can help control autoimmune conditions. They are administered orally, intravenously, by inhalation, or topically for a period of maximum 3-4 days due to high risk of side effects. Fluid retention and lower extremity oedema, raised intraocular pressure and blood pressure, mood swings, weight gain with fat deposits in the abdomen, easy bruising and slower wound healing are the main side effects of systemic administration. [90] Topical corticosteroids application on the throat surface may cause coughing, dry throat and red sores in that area. [91] Despite numerous scientific studies gathering evidence that systemic or topical antibiotic or anti-inflammatory treatments offer little or no benefit to most patients with throat infection, antibiotics are globally overprescribed for URT conditions with an increasing risk of bacterial resistance. [92-93]

Alternative treatments

Despite limited and conflicting evidence available in the literature, the use of alternative or natural remedies is on the rise, reflecting the poor efficacy of currently available drugs to treat nasopharyngeal infection. Some of the most popular yet controversial remedies are zinc lozenges and echinacea.

Zinc

Several mechanisms by which zinc may be effective against throat infection have been considered. At a concentration of about 0.1mmol/L, zinc blocks *in vitro* rhinovirus replication by preventing viral capsid protein formation. [94] It may also have immunomodulating properties, inducing production of interferon [95] to halt bacterial and viral growth. Zinc's cost and potential for side effects (unpleasant taste, mouth irritation, and gastrointestinal disturbances) are minor, but, as some clinical studies showed only a very slight reduction in duration of cold symptoms, zinc lozenges should rather be used as a complementary remedy, safe for short-term use in adults.

Echinacea

Echinacea is acquiring some reputation as remedy for common cold and throat infection in the US. This herb supposedly exerts its action through nonspecific immunomodulatory properties. Data from well-designed clinical trials supporting its efficacy are scarce, yet echinacea is used extensively. Early treatment initiation may decrease the severity and duration of acute respiratory infections [96] but data with standardized dosages and formulations are needed to conclusively recommend it as common cold treatment. Echinacea appears to be generally free of toxic side effects [97] but a theoretical risk of nonspecific stimulation of the immune system excludes its use by people with autoimmune disorders or receiving immunosuppressant drugs, as well as in HIV positive patients, patients with progressive systemic diseases, such as tuberculosis and multiple sclerosis, or with a known allergy to plants of the *Asteraceae* family. [98-99]

Vitamin C

Large doses of vitamin C are widely believed to prevent colds or relieve symptoms. However, several large-scale, controlled studies conducted in pediatric and adult populations to ascertain this popular theory failed to generate conclusive evidence that the vitamin lessens severity or duration of symptoms. [100] It should actually be used with caution, especially in the eldest and the youngest, as prolonged use in large amounts may cause severe diarrhea.

Honey

Honey has been traditionally used for the treatment of wounds, and to soothe coughs or sore throats. [101] Application of honey on the throat may help reduce local irritation as well as exert some osmotic effect to detach microbial contaminants. Honey has also been shown to possess antioxidant properties, probably owing to its high vitamin and total polyphenolic content. It was further observed to act as an antibacterial against *S. aureus* and *E. coli,* supposedly thanks to generation of H_2O_2 or to its lysozyme content. [102]

Although honey's antiviral capacities are still debated, its antimicrobial and soothing properties, absence of bacterial resistance and easy availability, make it a product of choice for minor throat infections. [103]

Gargles with hypertonic saline or sea water

Sore or strep throat usually involves microbial colonization of the pharyngeal mucosal surface, causing inflammation and erythema. Gargling with a solution that is saltier than the body fluids (i.e. a hypertonic solution) should generate osmosis and help clean the throat surface. The underlying principle is that if a semi-permeable membrane separates dilute and concentrated solutions then the dilute solution permeates through the membrane into the concentrated solution, the process going on until the concentrations are equal on both sides. Salt water is more concentrated than the water inside bacteria and will draw out water from the bacteria, leading to their dehydration and death through plasmolysis. [104] Besides osmotically inducing lysis of bacteria, the outward exudation of hypotonic liquid also helps to detach bacteria and reduce their concentration. Additionally, salt water gargling helps to wash away excess mucus and increase blood flow to the throat. Dilation of capillaries allows for faster circulation of infection-fighting cells. Another benefit of salt water is that it helps neutralize acids in the throat, restoring the natural pH balance that had been disrupted in the sore throat. As a consequence, the burning sensation is relieved and the mucous membranes become less irritated, which helps speed up the healing.

Unfortunately, the hypertonic properties of water containing 3-3.4% salt are not strong enough to detach all the contaminants from the throat surface, whereas increasing that concentration would cause strong irritation. Salt water therefore provides temporary relief but requires frequent gargles (4-5 per day), which is not very practical. [105]

Future developments
Although *in-vitro* some substances demonstrated activity against viruses implicated in URT infections, when tested in live patients their effectiveness proved unsatisfactory, probably because of the high antigenicity of the viruses involved. An emergent pharmacological target consists in a recently isolated cellular receptor responsible for cell attachment common to most rhinovirus serovars: the intercellular adhesion molecule (ICAM-1). Tremacamra, a recombinant soluble form of ICAM-1, has been investigated for its capacities to inhibit URT viruses' adhesion to mucosal cells but clinical results are inconclusive. [106]

Limitations of Antiviral Drugs
As mentioned above, clinical use of currently available antiviral drugs is limited to intracellular virus growth inhibitors that unfortunately present noxious side effects. There are no topical antivirals with the exception of a few topical Gp inhibitors recently authorized for topical use in Europe. [107-108] Besides, current antiviral drugs also present some shortcomings: high selectivity comes with the drawback of a restricted activity spectrum, [109] whereas viruses with the ability to lie dormant (such as *Herpesviridae* or *Retroviridae* viruses) can, in their latent state, evade antiviral therapy which targets active replication processes. [110-111] Furthermore, while antiviral treatment should be initiated as soon as possible to prevent tissue damage, correct early diagnosis or viral infections is often elusive. [76-77] Finally, antivirals, as all antimicrobial agents, are susceptible to drug resistance, [112-113] as illustrated by highly drug-resistant mutant HIV strains in some AIDS patients, in whom acyclovir-resistant HSV or VZV strains have also been detected. [114] This points out the dire need for alternative approaches in antiviral drug design so as to circumvent such limitations. Scientific development of newer molecules with

a greater efficacy, finer targeting of virus-specific functions, conceivably better safety profiles, may thwart resistance of mutants and allow greater virus inhibition, but the treatment of viral throat infection, involving symbiotic activity of viruses and bacteria on the throat surface, still remains a relatively uncharted scientific R&D field.

New virus glycoprotein inhibitors
Of all antimicrobial treatments available, gargling with hypertonic salt water or sea water (solutions containing 3 to 3.4% salt) has been found to be the most effective and safe treatment to minimize the amount of free virus particles, bacteria and other contaminants on the throat surface. However, despite their reasonable efficacy and good safety profile, such hypertonic saline solutions aren't used often because they prove too irritating, lack filmogen action, have a short-lived or limited efficacy, and cannot be patented. Increasing the salt concentration may have proved effective but a concentration above 3.4% salt in water induces the liberation of fucose, metacholine and histamins which are too highly irritant to be tolerated by the throat mucosa. [115]

Therefore, a French laboratory (Vitrobio) identified a non-irritant, cell-friendly, glycerol-type solution called VB-Gly, 7 times more osmotically active than sea water. [116] An improved version of this solution, with enhanced film retention capabilities, was invented and patented by this laboratory in 2013. [117] Through its high osmotic activity, VB-Gly induces instant exudation of hypotonic fluids across the mucosal surface of the throat, thereby cleaning the entire surface of all contaminants present, including viruses & bacteria, and acting as an instant, natural antiseptic, antimicrobial, and hydrating solution. Vitrobio scientists also observed that plant tannins are very big, inert plant molecules which have a strong affinity for proteins and other macromolecules [118] and can therefore bind to viral Gps such as the H1 and N1 on the influenza virus capsid. [119-120] Tannin–protein binding being specific, multiple experiments were conducted by incubating several plant tannins, or their specific fractions such as procyanidins (PCDs), with variable virus concentrations to evaluate tannin–virus Gp binding. [121] Finally, the researchers selected specific tannins or tannin fractions capable of binding with any one of the virus surface Gps as a new hypothesis to neutralize the influenza virus on the throat surface. Once a free virion binds with tannin, that virus particle cannot enter the cells anymore and, as a result, progression of the viral infection is stopped. Similar experiments were conducted to find tannins capable of binding and neutralizing virus entry-enhancing proteases found on the infected throat surface. [14] Neutralizing viruses and virus entry-enhancing proteases constitutes the best solution to stop virus infection without any cellular interaction on the pharyngeal mucosa. This specific tannin combination was then incorporated in the hypertonic VB-Gly solution for topical application to treat multiple viral diseases such as labial herpes, [122] genital herpes, [108] and rhinosinusitis. [107] In addition, the proteases involved in facilitating influenza virus entry into throat cells were also identified and similarly neutralized with specific plant tannin fractions. [14] The final selection of tannins incorporated in VB-Gly to treat throat infections was designed as a topical throat spray, totally safe and perfectly suited for application as a thin film on the throat surface. Owing to the filmogen properties of VB-Gly, the product film remains on the throat surface over a longer period of time, allowing sufficient time

for tannin-virus or tannin-protease binding, followed by their expulsion through hypotonic liquid exudation, the flow of which also detaches and eliminates other contaminants from the throat surface within a few minutes. Exerting its antimicrobial effect mechanically, such a treatment does not pose any risk of bacterial or viral resistance, and the absence of any pharmacological, biological, metabolic, or cellular interactions with the underlying cellular layer, guarantees the absence of possible toxic side effects. The product film also protects the throat's surface from dryness, irritation and external aggressions, thus contributing to reducing pain.

Clinical efficacy of new virus Gp and protease inhibitors

Because of difficulties in quantifying the exact number of virus-infected cells on the throat surface (due to swallowing and throat mobility), it was decided that a clinical trial to evaluate the efficacy of topical antivirals should initially be conducted in adult men and women (n=60) suffering from labial herpes, using herpes surface gB and gC Gp-inhibiting tannins. [122] A few drops of product (anti-herpes tannins in VB-Gly solution) were directly applied on the open herpes lesions (3-4 applications per day) for a maximum period of 14 days. Virus-infected cells were collected from the lesion with a swab before treatment and then 2 hours, and 4, 7 and 14 days after the start of treatment. Virus amount was quantified using Tzanck test. Results showed above 750 ($\pm$ 17.72) virus-infected cells in each lesion at the start of treatment. Just 2h after first drug application, the quantity of free virions was diminished by 38% (465 $\pm$10.82) indicating that the test product eliminates virus from the open lesions. Reduction in virus concentration inside the lesion reached 52% after 4 days of treatment, 70% after 7 days and 100% after 14 days of treatment. As increased liquid exudation was observed during the first 5-10 minutes following each product application, it was postulated that while the tannins bind the free virus particles, the osmotic imbalance caused by the VB-Gly base resulting in an outward flow of hypotonic liquid from the lesion drains the conjugated virus particles from the lesion. This clinical outcome, added to previous *in vitro* results, proves that tannins effectively bind the virus surface Gps and stop new virus infection.

To analyze the clinical efficacy of topical virus Gp-inhibiting tannins in VB-Gly against viral throat infection, a clinical trial was then conducted with a product containing influenza virus-neutralizing tannins (VB-Th4) in patients suffering from acute influenza-associated sore throat. 60 patients (adult men and women) were treated with VB-Th4 spray over a period of 14 consecutive days (3-4 applications per day) while 43 patients in the control group received other commonly used treatments. [123] Variations in total bacterial count on the throat surface was measured by collecting throat swabs and counting the number of colony-forming units (cfu/cm^2), before 1st treatment, 2h after 1st treatment, and then on days 4, 7, 10 and 14 or up to complete recovery. Throat pain, local throat irritation and erythema were also measured, on a 0 to 10 scoring scale, to evaluate clinical signs caused by bacterial infection. Complete haematological analyses, blood biochemical parameters, renal function tests were also performed at the start and at the end of the study to exclude any eventual possibilities of systemic interference. Control group patients were asked to take any treatment prescribed by their clinical ENT specialist and were evaluated similarly to patients treated with VB-Th4. Participants in both groups were authorized to take systemic antibiotics if found necessary by the investigator. Results indicate that on day 1, before treatment as well as 60 min after 1st application of VB-Th4, all patients were positive for bacterial throat infection. However, only 20/60 patients on day 4 and 17/60 on day 7 showed presence of bacteria above the normal limits. All patients had a normal bacterial count from day 10 onwards. The number of bacteria measured in throat swabs before VB-Th4 application exceeded the counting limits of 1950 ($\pm$179.43) cfu/cm^2. As soon as 2h after 1st product application the mean bacterial count was reduced to 1887.2 ($\pm$127.28) cfu/cm^2; then the values went down to 745.6 ($\pm$ 39.84) cfu/cm^2 on day 4 and 374 ($\pm$ 39.84) cfu/cm^2 on day 7, with normal values (50-100 cfu/cm^2) from day 10 onwards. Progressive and significant reduction was observed in throat pain, redness and irritation compared to the patients receiving other treatments. The number of patients who stopped all treatments after 2 days because they felt they had completely recovered represented 31% in the VB-Th4 group (n=60) compared to only 11% in the control group (n=43) treated with antiseptic sprays (28/43), salt water gargles (13/43) or expectorants (2/43). On the 7th day of treatment, 61% participants in the VB-Th-4 group stopped treatment due to recovery, compared to 25% in the control group. On day 10 almost all the patients (95.0%) in the VB-Th4 group had stopped treatment (57/60) compared to 28/43 patients (65.1%) in the control group. These results correspond to the absence of bacterial infection observed in most patients right after the 2nd day of treatment. During the 14-day study period, only 4/60 patients (6.66%) in the VB-Th4 group required antibiotherapy for an average duration of 7.1 day compared to 14/43 patients (32.56%) in the control group for an average period of 9.8 days. No topical or systemic side effects or any undesirable reaction were observed in any of the patients. None of the haematological, blood biochemical, or renal parameters was affected in the VB-Th4 group, indicating that the product's mode of action remains totally topical and mechanical.

Viral throat infection, accompanied by secondary bacterial infection, remains one of the most prevalent health problems in the world. [124] Although this condition is rarely mortal for the patients, it has a considerable socio-economic impact. In spite of tremendous medical progress, there is, currently, no effective topical antiviral available in the world. Almost all antiviral drugs are intended to stop virus growth at the intracellular level but have no effect on free virus particles present on the throat surface and are therefore of little or no use to treat topical viral infections where almost all the virus is active on the infected surface. As these antiviral drugs act intracellularly by modifying one of the mechanisms essential to cell survival, they stop viral growth but at the same time alter normal cellular functions, thus inducing various side effects. Viral and bacterial resistance to all currently available treatments is the second biggest concern for all virus-induced throat infections. [125] Other, less harmful, treatments, such as salt water gargling, only help reduce the amount of contaminants on the throat surface but cannot stop the infection totally and therefore may only be used to minimize clinical symptoms.

Recently developed virus glycoprotein inhibitors incorporated into an osmotically active hypertonic solution for topical application, conceived only with natural and non-toxic ingredients and capable of instantly eliminating viruses

and other microbial contaminants from the throat surface represent the safest and most logical scientific approach. Hopefully, those newly conceived drugs, based on this innovative concept, will contribute to offer a multifactorial topical treatment for virus-induced throat infections.

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