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The future of antivirals: broad-spectrum inhibitors

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Abstract

Purpose of review. Potent antivirals are successfully used for the treatment of infections with herpesviruses, hepatitis B and C virus and HIV and with some success for influenza viruses. However, no selective inhibitors are available for a multitude of medically important viruses, most of which are (re-)emerging RNA viruses. Since it is impossible to develop drugs against each of these viruses, broad-spectrum antiviral agents (BSAA) are a prime strategy to cope with this challenge.

Recent findings. We propose four categories of antiviral molecules that hold promise as BSAA. Several nucleoside analogues with broad antiviral activity have been described and given the relatively conserved nature of viral polymerases, it may be possible to develop more broad-spectrum nucleoside analogues. A number of viral proteins are relatively conserved between families and may also be interesting targets. Host-targeting antiviral drugs such as modulators of lipid metabolism and cyclophilin inhibitors can be explored as well. Finally, the potent and broad antiviral function of the immune system can be cooperated to develop immune-modulating BSAA.

Summary. Despite the recent advances, the BSAA-field is still in its infancy. Nevertheless, the discovery and development of such molecules will be a key aim of antiviral research in the coming decades.

Keywords: antiviral therapy; broad-spectrum antiviral agents; nucleoside analogues; host-targeting antivirals; immune modulation.

Introduction

Several epidemics of (re-)emerging viruses startled the world during the last decennia. The recent Ebola virus outbreak in West-Africa and the MERS epidemic in the Middle East and South Korea made clear that antivirals are urgently needed against a number of viruses and viral families for which no such drugs are available. Indeed, potent antivirals (or combinations thereof) are only available to treat infections with a limited number of viruses, i.e. herpesviruses, hepatitis B and C virus (HBV, HCV), HIV and to some extent for influenza virus. For many other viruses, including neglected and/or emerging RNA viruses that are sometimes highly pathogenic, like the ebolavirus, there are no antiviral treatment options. Furthermore, it can be expected that new, potentially pathogenic viruses will emerge in the future. Because it will not be economically viable to develop specific drugs for each individual virus, the development of broad-spectrum antiviral agents (BSAA) is believed to be essential to address the challenge of viral infections, both common and (re-)emerging. A first major and probably feasible achievement would be the development of molecules with broad antiviral activity within one virus family (pan-picornavirus, pan-alphavirus etc.). However, the 'holy grail' would ultimately be the discovery of antiviral molecules that target more than one virus family, or even all RNA viruses or all enveloped viruses. The development of BSAA may be focused on targeting a viral protein, but could also modulate a host cell factor. In this review, we will discuss possible strategies to develop broad-spectrum antiviral agents and highlight promising candidates and strategies.

Direct-acting broad-spectrum antiviral agents

Nucleoside and nucleotide analogues

Modified nucleosides and nucleotides have been among the earliest marketed antiviral drugs [1]. They are the cornerstone of anti-HIV therapy (e.g. tenofovir, emtricitabine, lamivudine,…) and crucial for the treatment of herpesvirus infections (aciclovir, ganciclovir, cidofovir,…) [1]. Some nucleoside analogues exert antiviral activity against an elaborate spectrum of viruses. Ribavirin is probably the

most well-known antiviral nucleoside with a broad antiviral activity and is used to treat chronic hepatitis C and E, respiratory syncytial virus (RSV) infections and with some success for the treatment of Lassa fever and hantavirus infection [2–6]. Secondly, several 2'-*C*-methylated nucleosides that had been discovered as HCV inhibitors were found to exert antiviral activity against several positive-sense RNA viruses. Sofosbuvir (a prodrug form of 2'-deoxy-2'-α-fluoro-β-C-methyluridine) is now successfully being used for the treatment of HCV infections but has less pronounced or no activity against other RNA viruses [7,8]. By contrast, the prototype inhibitor 2'-*C*-methycytidine inhibits a rather broad-spectrum of positive-sense RNA viruses including flavi-, noro- and picornaviruses [9–11]. Moreover, favipiravir, also known as T-705, has antiviral activity against both positive- and negative-sense RNA viruses [12]. This molecule is regarded as a nucleobase and is intracellularly metabolized to a nucleotide analogue that inhibits the influenza RNA-dependent RNA polymerase. The compound has been approved in Japan for the treatment of influenza virus infections. Similarly, the adenosine analogue BCX4430 acts as a chain terminator of RNA virus polymerases and elicits *in vitro* antiviral activity against most positive- and negative-sense RNA viruses. BCX4430 is most potent against filoviruses and protective activity has been demonstrated in filovirus-infected primates [13]. Nevertheless, these drugs exhibit either toxicity and strong side effects (e.g. 2'-*C*-methylcytidine, ribavirin), limited clinical efficacy against some viruses (e.g. ribavirin against RSV infections) and/or they are still in (early) development [14–16]. Finally, the spectrum of susceptible viral species is often too limited for these drugs to be considered true BSAA.

Despite the drawbacks and limitations of the currently available nucleoside analogues, this class of molecules still holds great promise to deliver *bona fide* BSAA. The replication of the viral genome is an essential part of the viral life cycle and consequently most viruses encode their own polymerases which are significantly divergent from their mammalian counterparts. There is a certain degree of conservation within each type of polymerase family (e.g. DNA- or RNA-dependent,… [17]), suggesting the possibility to develop BSAA that inhibit virtually all polymerases of a certain type. To identify lead molecules for further development into such nucleoside-based BSAAs, a tailored screening approach

may be quintessential. An enormous collection of nucleoside and nucleotide analogues has been synthesized to date [18]; a first step in a successful screening campaign would therefore be the rigorous selection of a high-end nucleoside library based on criteria such as structural diversity, drug-like characteristics, cell-permeability, solubility, etc. This library can subsequently be tested using a specifically designed screening procedure. A first possibility for such screening would be to use high-throughput enzymatic polymerase assays [19,20] where all selected molecules are tested against several representative viral polymerases of a certain type (e.g. RNA-dependent RNA polymerases from different virus families), searching for molecules that inhibit all tested enzymes. Another approach might be to evaluate such libraries in phenotypic cell-based antiviral assays against a panel of viruses that are representative for particular genera, families or groups of viruses. A third strategy is the rational design of nucleotide analogues based on a comparison of available polymerase crystal structures and specifically the conserved features in the catalytic site. Further development of hit molecules would include expanding the number of tested viruses and chemical modification of the initial hit (so-called hit explosion) to increase antiviral potency and the number of susceptible agents, but also to improve selectivity and pharmacokinetic parameters. Since some of the DNA virus-targeting nucleoside analogues already have a rather broad-spectrum of activity, these efforts should probably be directed towards RNA viruses initially.

Viral protease inhibitors

Besides the viral polymerase, the viral protease is one of the most studied antiviral targets and virus-specific protease inhibitors are used successfully to treat HIV and HCV infections. The development of protease inhibitors targeting multiple virus families might be an interesting strategy. Based on phylogenetic analysis, picornaviruses, caliciviruses and coronaviruses can be classified in the picornavirus-like supercluster [21]. These viruses all have 3C or 3C-like proteases with a typical chymotrypsin-like fold and a catalytic triad (or dyad) containing a cysteine residue as a nucleophile, making them interesting targets for BSAA development. Rupintrivir for instance is an irreversible 3C-

protease inhibitor that was originally developed for the treatment of human rhinovirus infections [22] and antiviral activity has also been shown against other picornaviruses, coronaviruses and norovirus [23–25]. Other inhibitors of 3C-like proteases were reported with broad-spectrum antiviral activity against both feline coronaviruses and caliciviruses [26]. Therefore, the design of BSAA targeting proteases of different virus families appears feasible and could be a good strategy to develop broader-spectrum antivirals.

Host-targeting antivirals (HTA) as BSAA

Targeting a host protein that is essential in the viral life cycle of different virus families could be a second attractive strategy for broad-spectrum antiviral intervention. However, this strategy is still somewhat controversial since inhibiting the primary roles of host factors may result in toxicity or adverse effects and polymorphisms in a host factor and variable expression levels between patients might be problematic. On the other hand, the selection of viral resistance, which is a major problem for conventional virus-specific drugs, is probably lower for a HTA. The feasibility of HTA strategies will likely depend on which host factor is targeted and how the viruses and the cell depend on its function(s). Modulators of the host lipid metabolism, nitazoxanide and cyclophilin inhibitors are host-targeting antivirals that are considered as potential BSAA.

Modulators of lipid metabolism

Many viruses are highly dependent on the host lipid metabolism for replication [27]. The lipid metabolism is therefore considered a prime target for BSAA. One of the most widely used classes of lipid modulators are the cholesterol-lowering statins. Statins inhibit the 3-hydroxy-3-methyl-glutaryl coenzyme A reductase, the rate-limiting enzyme involved in cholesterol biosynthesis in the liver. Statins have been reported to possess *in vitro* antiviral activity against a variety of viruses, such as HCV, HIV, poliovirus, cytomegalovirus, dengue virus and RSV [28–35]. Nevertheless, studies evaluating the antiviral efficacy of statins in patients yielded contradictory results. In HCV-infected

patients for instance, a modest or even no antiviral effect of statins was observed when used as a monotherapy. However, in combination with the previous standard of care (pegylated interferon-α and ribavirin) a significant increase in sustained virological response rates was observed [36,37]. The potential use of statins as BSAA will require more study to prove its *in vivo* antiviral efficacy.

Arbidol is another lipid modulator that is approved in China and Russia for the prophylaxis and treatment of influenza and other respiratory viral infections. This indole derivative also inhibits the replication of many other enveloped and non-enveloped RNA and DNA viruses, including HBV, HCV and chikungunya virus [38]. Recent studies suggest that arbidol has a dual mechanism of action: it binds to lipid membranes and interacts with aromatic amino acids in the viral envelope glycoprotein [39]. In this way, it interferes with viral entry and membrane fusion. Arbidol combines good bioavailability with a safe record of use in patients, making it a promising BSAA candidate [40]; however, *in vivo* studies confirming its antiviral effect are only sparsely available.

Finally, LJ001 is a lipophilic thiazolidine derivative that is effective against several enveloped viruses including influenza virus, HIV and filoviruses [41]. In contrast, the compound has no effect on non-enveloped viruses. LJ001 targets the viral lipid envelope and hampers its ability to mediate virion-cell fusion. Recent studies showed that LJ001 induced lipid oxidation, thereby negatively impacting the biophysical properties of membranes (such as curvature and fluidity) needed for viral fusion [41]. Unfortunately, LJ001 itself is unsuitable for further development because of its poor physiological stability and the requirement of light for its antiviral mechanism. New analogues have been developed to overcome these negative characteristics [42]. These analogues have improved antiviral activity, better pharmacokinetic characteristics and altered light-absorbing properties. However, when evaluated in a mouse infection model of Rift Valley fever virus, these molecules were only able to delay the time of death [42]. Although this particular class of molecules seems less suitable for clinical development, the viral membrane could still be a viable target for BSAAs that disturb viral-cell fusion [43,44]. Squalamine is another compound that targets the host membrane, but its mechanism of action differs from LJ001 and analogues. Squalamine is a compound isolated from the dogfish

shark and the sea lamprey that inhibits enveloped RNA and DNA viruses both *in vitro* and *in vivo* [45]. The mechanism of antiviral activity is proposed to be the neutralization of the negative electrostatic surface charge of intracellular membranes, thereby making the cellular environment less favorable for viral replication. This disruption of electrostatic potential does not result in structural damage of cellular membranes, as measured by changes in cell permeability [46]. As squalamine can be readily synthesized [47] and has already been studied in humans in several phase 2 clinical trials for cancer and retinal vasculopathies without serious adverse events [48], its potential to be used as a BSAA could be further explored.

Nitazoxanide

Nitazoxanide was originally developed and commercialized as an antiprotozoal agent and was licensed in the USA as an orphan drug for the treatment of diarrhea caused by *Cryptosporidium parvum* and *Giardia intestinalis* [49]. It is also widely used in India and Latin-America to treat intestinal parasitic infections. In addition to its anti-parasitic activity, nitazoxanide inhibits a broad range of unrelated RNA and DNA viruses [50]. Nitazoxanide inhibits the influenza virus by blocking the maturation of the viral hemagglutinin at the post-translational stage [51]. In HCV-infected cell cultures, nitazoxanide activated protein kinase R, an important component of the innate immune system [52]. The antiviral efficacy of nitazoxanide has also been evaluated in patients. A phase 2b/3 clinical study in patients with laboratory-confirmed influenza showed that nitazoxanide decreased the duration of clinical symptoms and reduced viral shedding compared to placebo [53]. A large phase 3 trial is ongoing. Phase 2 studies also demonstrated that nitazoxanide significantly reduced the duration of symptoms in patients infected with rotavirus or norovirus [54]. For HCV-infected patients, clinical studies showed improved responses when nitazoxanide was combined with pegylated interferon [55]; however, the clinical development for HCV treatment was discontinued due to the recent approval of direct-acting antivirals. The broad-spectrum antiviral activity together with the high barrier for resistance and proven *in vivo* efficacy for some viral infections make

nitazoxanide an attractive candidate to be developed as a BSAA. Initially, clinical development of this drug will primarily focus on viral respiratory infections and gastroenteritis.

Cyclophilin antagonists

The cyclophilins are peptidyl-prolyl cis/trans-isomerases that are required for the proper folding of certain host proteins [56] and also to play an important role in the life cycles of diverse viruses [57]. Cyclosporine A and sanglifehrin A are immunosuppressive molecules that inhibit cyclophilins [58]. By chemical modification, analogues were generated without immunosuppressive properties. These cyclophilin inhibitors inhibit a broad range of RNA and DNA viruses, both *in vitro* and in animal models [57]. The most advanced molecules are alisporivir and SCY-635. These molecules demonstrated therapeutic efficacy in HCV-infected patients [59,60]. Mechanistically, cyclophilin A (CypA) was shown to interact with the HCV NS5A protein [61,62] and multiple mutations in NS5A were required to confer *in vitro* resistance to alisporivir, suggesting a high barrier to resistance [62,63]. For HIV, it was reported that CypA binds to the capsid protein p24 [64]. Although cyclophilin inhibitors potently suppress HIV infection *in vitro* and in the majority of patients, naturally pre-existing capsid variants resistant to treatment were observed and preclude the broad therapeutic use of cyclophilin inhibitors against HIV [65,66]. However, as cyclophilins are indispensable for the replication of many viruses, these proteins may be an interesting host target for the development of BSAA. Furthermore, CypA knockout studies in a human cell line and in mice showed that CypA is not essential for basic cell survival [67,68], countering concerns for associated cellular toxicity. However, the *in vivo* efficacy of cyclophilin inhibitors will need to be demonstrated for other viruses.

Immune modulation

Lastly, the immune system can be regarded as the most broad-spectrum antiviral mechanism currently known. By combining innate and adaptive immune mechanisms, most microbial infections can be cleared successfully. However, in particular cases, the immune system is unable to eliminate

the pathogen (e.g. chronic viral hepatitis, herpesvirus latency, HIV infections,…) or its actions may be damaging the host (for instance during RSV infection [69]). Many viruses circumvent or even cooperate the immune response for their own benefit [70]. Therefore, specific modulation of the immune response is an attractive strategy to address these persistent, latent or immune-evading viruses, but also to develop novel and potentially broad-spectrum antiviral therapies. The current clinical practice already includes a form of immune modulation-based antiviral therapy: (pegylated) interferon used to be an important part of chronic viral hepatitis therapy, although it may have severe side effects.

Immune modulation as an antiviral therapy has distinct advantages, such as the possibility to target a wide array of viruses and other pathogens and a decreased risk for resistance development. However, there may be certain risks when modulating such a complex system, for instance the possibility to induce auto-immunity or cytokine storms, unwanted effects on commensal microbes or exacerbation of inflammatory diseases. This highlights the need for a very thorough understanding of the underlying immune mechanisms and extensive safety testing.

Modulation of innate immune signaling would be an interesting alternative for the direct use of interferon. One strategy employs the RIG-I agonist 5'pppRNA [71,72]: this double-stranded RNA oligomer activates several innate immune pathways and has broad-spectrum antiviral activity against multiple DNA and RNA viruses. In addition, it protects mice from lethal influenza virus infection [71]. Nevertheless, 5'pppRNA still requires parenteral administration. The use of small molecules that trigger an interferon-response provides an interesting possibility to overcome parenteral administration, like for instance compound C3 [73].

An alternative and more specific approach is to selectively activate certain factors of the interferon effector system, thus obtaining a more targeted response and possibly avoiding interferon's side effects. One example are RNase L-activating molecules with a broad activity against RNA viruses [74]. GSK983 is another small molecule that induces a specific subset of interferon-stimulated genes and has antiviral activity against a number of unrelated viruses, although not all pathogens are

susceptible [75]. Finally, two very attractive targets for the development of activating molecules may be the IFIT (interferon-induced protein with tetratricopeptide repeats) and IFITM (interferon-induced transmembrane protein) protein families which display broad-spectrum antiviral activity [76], but no such activating molecules have been described yet.

Another interesting development in antiviral immune modulation are double-stranded RNA (dsRNA)-activated caspase oligomerizers (DRACOs) [77]. These engineered proteins comprise a dsRNA-detection domain, an apoptosis-induction domain and a transduction tag for cellular delivery. Most viruses (including DNA viruses) produce long dsRNA during their replication cycle which is recognized by the dsRNA-detection domain. By fusing this detector to an apoptosis-inducing domain, DRACOs selectively eliminate virus-infected cells without harming non-infected cells. Consequently, these constructs have antiviral activity against a large spectrum of DNA and RNA viruses [77,78]. An *in vivo* proof-of-concept was provided in an influenza mouse model [77]. However, concerns regarding safety and specificity, delivery, cost and *in vivo* efficacy need to be addressed before DRACOs can advance into clinical trials.

Priorities for future research in antiviral immune modulation thus include (1) the discovery and development of more potent molecules with an extensive spectrum of susceptible viruses, (2) the *in vivo* validation of the available and novel molecules against clinically relevant pathogens and (3) the minimization of toxicity and side effects. A detailed understanding of the fundamental mechanisms governing the innate immune response is imperative in this regard.

Conclusion and perspectives

The past decades, antiviral research has resulted in over 30 marketed antiviral agents, most of them targeting a specific viral species. To cope with the threat of other viral pathogens, particularly (re-)emerging and neglected RNA viruses, the development of BSAA is critical. We discuss different approaches to obtain such molecules, i.e. focusing on nucleoside analogues, inhibitors of other conserved sites in viral enzymes, host-targeting antivirals and finally immune modulators. This list is

not exhaustive; other interesting strategies may emerge as our knowledge on viruses expands. Future research efforts should focus on validating these and other approaches and on the early development of candidate BSAA, but they should also be directed at increasing the fundamental understanding of the viral life cycle as this will provide important insights for the development of new broad-spectrum antiviral strategies.

Key points

- Broad-spectrum antiviral drugs are a prime strategy to cope with the large number of medically important, neglected and/or (re-)emerging viruses.
- Since some nucleoside analogues have proven to target a broad range of viruses, further research into these molecules may yield even more potent and broad-spectrum inhibitors.
- Another strategy to reach a broad spectrum of activity is to target relatively conserved viral enzymes, such as the viral protease.
- Host-targeting antiviral drugs are a third approach, probably with a high barrier to resistance.
- Finally, immune modulation uses specific properties of the host immune system and is one of the most promising strategies for future antiviral therapy.

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Conflicts of interest

None

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Highlights:

13. Warren et al 2014: * Recent study describing a new broad-spectrum antiviral nucleoside analogue including in vivo testing against filoviruses.

43. Vigant et al 2015: ** A comprehensive review of the broad-spectrum potential of antivirals that target the membrane fusion process.

50. Rossignol 2014: * A complete account on the current knowledge on nitazoxanide, a repurposed broad-spectrum antiviral drug

72. Olagnier et al 2014: * An interesting study describing inhibition of arthropod-borne virus replication by the RIG-I agonist 5'pppRNA