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REVIEW

Antiviral activity of cationic amphiphilic drugs

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ABSTRACT

Introduction: Emerging and reemerging viral infections represent a major concern for human and veterinary public health and there is an urgent need for the development of broad-spectrum antivirals. **Areas covered**: A recent strategy in antiviral research is based on the identification of molecules targeting host functions required for infection of multiple viruses. A number of FDA-approved drugs used to treat several human diseases are cationic amphiphilic drugs (CADs) that have the ability to accumulate inside cells affecting several structures/functions hijacked by viruses during infection. In this review we summarized the CADs' chemical properties and effects on the cells and reported the main FDA-approved CADs that have been identified so far as potential antivirals in drug repurposing studies. **Expert commentary**: Although there have been concerns regarding the efficacy and the possible side effects of the off-label use of CADs as antivirals, they seem to represent a promising starting point for the development of broad-spectrum antiviral strategies. Further knowledge about their mechanism of action is required to improve their antiviral activity and to reduce the risk of side effects.

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1. Background

Infectious diseases still represent one of the major concerns for human and veterinary public health and, as a consequence, for the global economy. Due to climate and environmental changes, travel and trade globalization, infectious agents spread more rapidly and widely than in the last century [1], as showed by the number of emerging and reemerging viral infections appeared since the 2000, such as the Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV), the Middle East Respiratory Syndrome Coronavirus (MERS-CoV), the 2009 pandemic H1N1 influenza virus, the wide epidemic of Ebola virus (EBOV) in West Africa and currently by the potentially pandemic diffusion of Zika virus [1–5].

Moreover, additional zoonotic agents have been identified, most of them causing rare but potentially lethal infections in humans. Highly pathogenic viruses mainly belong to the *Bunyaviridae* (Crimean-Congo Haemorrhagic Fever virus, Rift Valley virus and several hantaviruses), *Flaviviridae* (Dengue, West Nile virus), *Arenaviridae* (Lassa virus and Junin virus), *Paramyxoviridae* (Hendra virus and Nipah virus), and *Orthomyxoviridae* (avian influenza viruses). Due to their sporadic outbreaks in humans, these highly pathogenic viruses require a continuous surveillance activity which needs a significant effort of the National Health Systems and of the International Authorities [6–10]. To date, no specific drugs are available to treat patients infected with most agents belonging to this wide array of the emerging and reemerging viruses. Thus, there is an urgent need for the development/validation of effective antivirals.

The classical approach to develop an antiviral drug is based on compound affecting the functions of specific viral proteins that play a key role in the viral life cycles. This approach has been used with success, for instance, to control the human immunodeficiency virus (HIV) infection. Indeed, thanks to the available treatments, AIDS has become a chronic disease and the life expectancy of people living with HIV is significantly increased over the years [11]. More recently, following a similar approach, effective drugs against the hepatitis C virus (HCV) have been developed [11]. Currently available antiviral drugs include more than 40 compounds that have been officially approved for clinical use [11]. However, the number of human health threatening viruses that can be controlled by these drugs is less than 10 [12]. The situation is even worst in the case of neglected viral infections, for whose treatment no selective drugs are expected to appear on the market in the near future, due to their limited business potential, and to the fact that usually they affect populations with poor socioeconomical background. Finally, viruses classified as highly pathogenic require biosafety level 3 or 4 facilities and protocols, thus rendering drug discovery and development extremely difficult.

Taking into account these aspects, along with the cost–benefit analysis to develop specific drugs for each virus and the problem of the selection of drug-resistant mutants, new approaches are focused on the identification of broad-spectrum compounds targeting mechanisms allowing the establishment of infection which are shared between different viruses.

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One strategy is represented by the development/selection of compounds able to affect several viruses, by inhibiting common viral enzymatic functions. The first example of such a drug was the ribavirin, followed by the more recent generation of new nucleotide and nucleoside analogs [12].

Viruses, as obligate intracellular parasites, hijack the cellular proteins and membranes during their replication cycle by exploiting several cellular components to allow viral entry, genome replication and viral particle assembly/budding. Indeed, agents targeting host functions required for infection of multiple viruses, in the absence of a significant cytotoxicity, could represent an alternative way to develop new broad-spectrum antiviral therapies [12]. In particular, the identification of commercially available drugs, already approved for the use in humans, and active against viruses, might accelerate the organization of clinical trials and eventually clinical use, especially in the case of outbreak with highly pathogenic viruses.

2. Cationic amphiphilic drugs

Cationic amphiphilic drugs (CADs) are a wide group of chemicals that are characterized by common structural features, that is, a hydrophobic aromatic ring or ring system and a hydrophilic side-chain containing an ionizable amine functional group. CADs include antidepressants, antibiotics, antipsychotics, antimalarial, antiarrhythmic, cholesterol-lowering and fertility-regulator drugs [13,14]. CADs have the propensity to interact with different cell membranes and accumulate in acidic intracellular compartments such as late endosomes/lysosomes (LE/Lys). The cellular uptake mechanisms markedly differ between different CADs. Indeed, they can accumulate into the Lys within minutes or hours after *in vitro* cell exposition and the different kinetic depends from the chemical-physical characteristics of the molecules [13,15–17]. The amine functional group of these compounds is mainly unprotonated at physiological pH. Once inside the acidic environment of the LE/Lys, the molecules become protonated and, since they cannot longer permeate the membrane, get trapped inside the organelle (Figure 1). This pH-dependent partitioning of CADs results in a pronounced accumulation of the drugs leading to various physiological and morphological alterations of the LE/Lys compartment. One of the effects observed is the accumulation inside the LE/Lys of various lipid species, such as cholesterol, sphingomyelin, phosphatidylserine, phosphatidylethanolamine, phosphatidylcholine, and bis (monoacylglycero)phosphate (BMP), inducing the accumulation of membranous structures and a marked expansion of the organelle volume. This phenotype recalls the one observed in patients with the Niemann Pick type-C (NPC) disease, a lipid storage disorder [13,14]. More than 50 CADs have been showed to induce phospholipidosis at therapeutically relevant concentrations and after chronic treatments [13,14]. However, CADs currently in clinical use are well tolerated and the lipid accumulation is not associated in a clear cut manner to clinical consequences [13,14]. Furthermore, cell changes induced by CADs are reversible and no rebound effects have been described associated to the discontinuation of CADs [13].

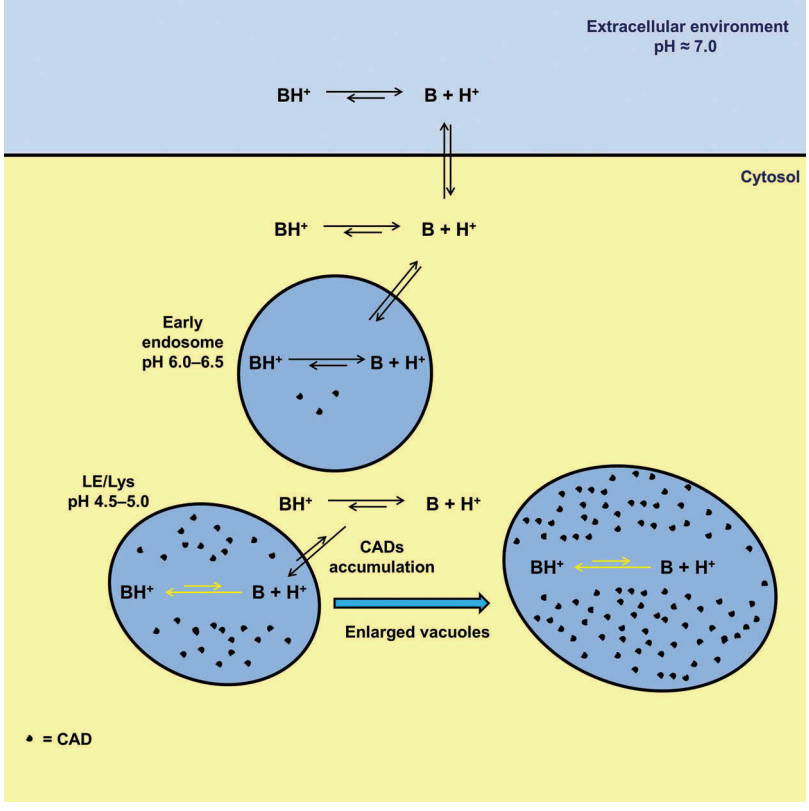


Figure 1. Lysosomal trapping of cationic amphiphilic drugs (CADs). CADs are weak bases (B) and they cumulate in intracellular acidic compartments because the lysosomal membrane is much less permeable to the charged protonated bases (BH+) compared to the uncharged form. Accumulation of CADs inside the Late Endosomes/Lysosomes (LE/Lys) induces an enlargement of the organelles creating large vacuoles.

Drug-induced phospholipidosis is believed to be the result of the direct interaction of CADs with membrane phospholipids and the ability of a specific CAD to induce phospholipidosis has been correlated positively with the strength of the drug/phospholipid interactions [14]. The interaction of CADs with membranes can modify their permeability as well as the membrane-proximal pH, thus affecting several biological processes in particular at the level of LE/Lys, such as the inhibition of several enzymatic activities and the change in the distribution of lysosomal enzymes [13,14,18–20]. The acid sphingomyelinase (aSMase), a lysosomal enzyme which catalyzes the hydrolysis of sphingomyelin into ceramide and phosphorylcoline, is one of the targets of several CADs [13,21]. It has been shown that the antidepressant desipramine and related drugs induce the detachment of aSMase from the inner membrane leaflet of Lys with its consecutive inactivation, resulting in the accumulation of sphingomyelin [13]. The reduction of the aSMase activity has been also reported for other two CADs: the antiarrhythmic amiodarone and the inhibitor of cholesterol transport U18666A [21,22]. Furthermore, it has been reported that structurally different CADs have an additive effect on the inhibition of aSMase activity, arguing for action of these compounds on the same molecular target [13]. Interestingly, a low level of aSMase activity is detectable in cells from patients with the NPC disease [13], further underlying the similarity between the cellular phenotype observed in cells of NPC patients and in those of individuals treated with CADs. The ability of CADs to interact with the biogenesis and luminal acidification of LE/Lys compartment may affect other proteases, such as phospholipases and catepsins, and could explain the ability of amiodarone to inhibit the degradation of lung surfactant protein A both *in vitro* as well as *in vivo* [15,20,23]. Furthermore, it has been shown that bepridil (an antiarrhythmic drug), amiodarone, and U18666A inhibit the activity of the lysosomal beta-secretase, while differentially modify the specificity of gamma-secretase to cleave the amyloid precursor protein [18,24]. It has to be noted that CADs do not necessarily interact with the phospholipid bilayers of the cellular membranes [15]. For example, the antimalarial drug chloroquine does not interact with phospholipids [15]. On the other hand, when this interaction takes place, it can have different features. For instance, while the antipsychotic drug chlorpromazine, which aspecifically interacts with membrane, causes an increased membrane permeability, amiodarone alters lipid dynamics by interacting with the hydrophobic core of the membrane bilayer [15]. These differences in CADs behavior give reason of their different ability in inducing phospholipidosis and cellular metabolism alterations.

Finally, it has been suggested that different CADs can induce the same cellular phenotype, by targeting specific substrates, but with a different mechanism of action. Indeed, U18666A and amiodarone induce a NPC-like phenotype with the formation of enlarged vacuoles enriched in BMP, cholesterol removal alleviates the change in BMP distribution induced by U18666A, but not the one induced by amiodarone suggesting a different mechanism of action [17].

3. Antiviral activity of CADS

Overall, deregulation of the endolysosomal pathway and lipid homeostasis mediated by CAD accumulation in the LE/Lys compartment affect several cellular activities, such as macro- and/or micro-pinocytosis, the organization of the membrane invagination systems, and the vesicular transport of material to the Lys [17,23,25–28]. The main driving force allowing CAD accumulation inside the LE/Lys compartment is the CAD trapping mechanism. Thus, CADs display pleiotropic effects in the cells targeting several cellular pathways/structures, some of which are important for viral replication. Several viruses require macropinocytosis to enter into the target cells [29] and the functionality of enzymes like aSMase and cathepsins can be required for an efficient viral internalization and/or for viral glycoproteins processing to activate their fusogenic potential [30–35]. In addition, lipids such as cholesterol and BMP can play a relevant role in viral entry, replication and budding [36–39].

As mentioned above, numerous small-molecular-weight compounds currently used as therapeutics for a wide range of human diseases are CADs. During the last decades, research activities focused on drug repurposing programs or on the characterization of viral biology/pathogenic mechanisms showed that several CADs display antiviral properties. The main FDA-approved CADs with antiviral activity are reported in Table 1 and will be described in the following sections of this review.

3.1. Antiarrhythmics drugs

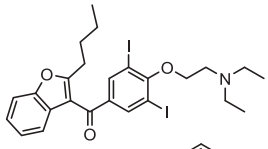
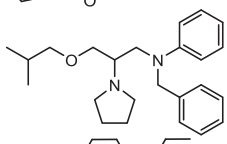
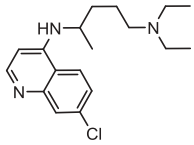
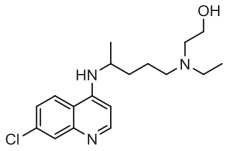
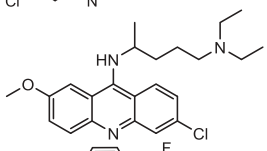
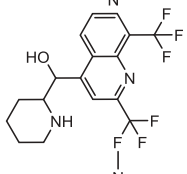
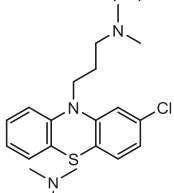
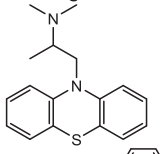
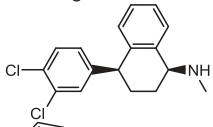
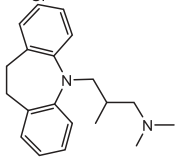
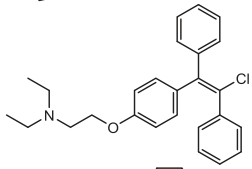
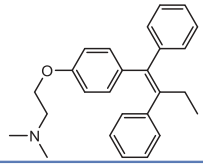
Amiodarone, dronedarone, and verapamil are ion channel blockers used for the control of supraventricular and ventricular arrhythmias. It has been recently shown that these molecules inhibit the cell entry of filoviruses [40,41]. In particular, it has been demonstrated that amiodarone and its main metabolite (methyldiethanolamine) show an additive effect inhibiting EBOV entry at concentrations close to those found in the sera of patients treated for arrhythmia [41]. The drugs act by interfering with the fusion of the viral envelope with the endosomal membrane and the antiviral activity is correlated with drug ability to accumulate into LE/Lys compartment and interfere with the endocytic pathway [41].

In vivo positive effects of amiodarone were also partially reported in a mouse model [42], while no significant clinical improvements have been reported in humans treated with amiodarone during the last EBOV epidemic in Western Africa [60–62].

Interestingly, amiodarone also inhibits the infection of additional viruses, that is, the New World arenavirus Guanarito, the SARS-CoV, and HCV [40,63,64]. However, viruses as the Old World arenavirus Lassa, the *Rhabdoviridae* (vesicular stomatitis virus – VSV, and rabies), *Bunyaviridae* (Hantaan and Crimean-Congo Hemorrhaging Fever virus – CCHFV) and Dengue are not inhibited, at least under the tested conditions [40,65,66]. An *in vitro* strong antiviral activity against EBOV has also been reported for the calcium channel blocker bepridil. As for the above-described drugs, bepridil inhibits a step which follows viral internalization while taking place before viral fusion. Furthermore, bepridil displays also a significant survival benefits with a 100% survival rate for mice exposed to Ebola virus [43].

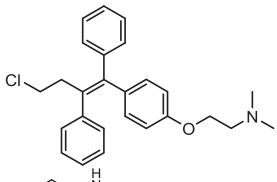
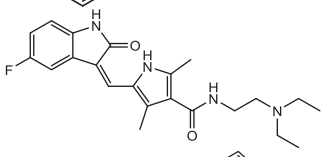
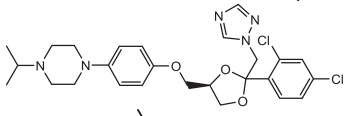
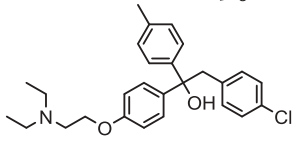
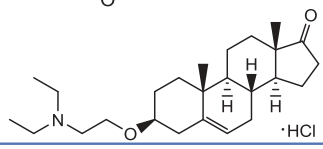
Although ion channel blockers generally inhibit viral infection at early stages of the life cycle, effects of amiodarone and verapamil on additional steps of viral replication following the

Table 1. Structure and antiviral efficacy of the main CADs.

CAD name	CAD structure	Antiviral efficacy	Ref.
Amiodarone		Filovirus – IC_{50} 0.25–1.38 μg/mL Ebola virus – IC_{50} 5.60 μM HCV – EC_{50} 2.10 μM	[40–42]
Bepridil		Ebola virus – IC_{50} 3.21–5.08 μM	[43]
Chloroquine		CCHFV – IC_{50} 28.00–43.00 μM Filovirus – EC_{50} 4.70–15.00 μM HCoV-OC43 – EC_{50} 0.306 μM KSHV – IC_{50} 3.30–5.10 μM MERS-CoV – EC_{50} 3.00–6.28 μM SARS-CoV – EC_{50} 6.54–8.80 μM	[44–49]
Hydroxychloroquine		Dengue virus type 2 – EC_{50} 9.70–12.90 μM KSHV – IC_{50} 1.30 μM	[47,50]
Quinacrine		Dengue virus type 2 – EC_{50} 7.09 μM Zika virus – EC_{50} 2.27 μM	[51]
Mefloquine		Dengue virus type 2 – EC_{50} 4.36 μM Zika virus – EC_{50} 3.95 μM	[51]
Chloropromazine		CCHFV – IC_{50} 10.80–15.70 μM MERS-CoV – EC_{50} 4.90–9.51 μM SARS-CoV – EC_{50} 12.97 μM	[44,48,49]
Promethazine		Filovirus – IC_{50} 19.10–19.40 μM	[52]
Sertraline		Ebola virus – IC_{50} 1.44–3.13 μM	[43]
Trimipramine		Filovirus – IC_{50} 10.90–11.10 μM	[52]
Clomiphene		Filovirus – IC_{50} 0.76–11.10 μM HCV – EC_{50}	[43,53,54]
Tamoxifen		HCV – EC_{50} 0.10 μM HSV – IC_{50} 4.89 μM MERS-CoV – EC_{50} 10.12 μM SARS-CoV – EC_{50} 92.89 μM	[49,54,55]

(*Continued*)

Table 1. (Continued).

CAD name	CAD structure	Antiviral efficacy	Ref.
Toremifene		Filovirus – IC_{50} 0.03–6.17 μM MERS-CoV – EC_{50} 12.92 μM SARS-CoV – EC_{50} 11.97 μM	[43,49,53]
Sunitinib		HCV – IC_{50} 0.05 μM	[56]
Terconazole		Ebola virus – IC_{50} 7.07–8.26 μM	[43,57]
Triparanol		Ebola virus – IC_{50} 1.92 μM	[57]
U18666A		Ebola virus – IC_{50} 1.60–8.00 μM Dengue virus – IC_{50} 2.90–6.20 μM HCV – IC_{50} 0.13 μM	[57–59]

entry phase have been also described [63,64]. For instance, in the case of HCV, amiodarone affects both viral entry, by downregulating the CD81 viral receptor on the cellular membrane, as well as viral assembly, via the suppression of the microsomal triacylglycerol transfer protein activity [64]. Verapamil has been reported to inhibit the egress of mature sindbis and vesicular stomatitis viruses from infected cells [67].

3.2. Antimalarial drugs

Chloroquine is an antimalarial drug that has been gradually dismissed from antimalarial therapy and prophylaxis due to the emergence of resistant *Plasmodium* strains. It has been shown that chloroquine (and its derivatives) inhibits the *in vitro* replication of several viruses including HIV, SARS- and MERS-CoV, alphaviruses, CCHFV, Dengue, Zika virus, Japanese encephalitis virus, EBOV, influenza virus, calicivirus, and Herpes simplex virus type-1 (HSV-1) [42,44,48,50,68–77]. Furthermore, it has been recently reported that chloroquine inhibits lytic replication, but not the latent infection of Epstein-Barr virus and Kaposis's sarcoma-associated herpesvirus in human B cells at clinically acceptable doses [47].

The mechanism of action of chloroquine is multiple, depending on the pathogen. It likely acts by increasing the endosome-Lys and Golgi vesicle pH, and by downregulating the production of cytokines (IFN-γ and TNF-α), and the expression of TNF-α receptor [78,79]. Thus, chloroquine can directly inhibit viruses that require an acidic pH inside the LE/Lys to enter into the cytosol and can mitigate the deleterious effect of the immune activation induced by certain viral infections. Unfortunately, *in vivo* evidence do not support, or poorly support, the efficacy of chloroquine in the treatment of HIV, dengue, EBOV and influenza [42,70,72,80–82]. Two clinical trials specifically aimed at addressing chloroquine efficacy on acute phase of chikungunya virus infection were concluded with contrasting results [69]. On the other hand, chloroquine seems to be effective *in vivo*, at least in the mouse model, in inhibiting the human coronavirus subtype OC43 and EBOV [45,46], as well as in the Aotus monkeys, affecting the dengue virus type 2 replication [83].

Among the antimalarial drugs, amodiaquine and its derivatives along with quinacrine and mefloquine have been shown to display an *in vitro* activity against flaviviruses [51].

3.3. Psychoactive drugs

The well-known antipsychotic drug chlorpromazine has been reported to have antiviral activity against adenovirus, EBOV, coronavirus, and CCHFV [44,48,49,84,85]. Chlorpromazine is known to interfere with the formation of clathrin-coated pits. Thus, it would act by inhibiting the clathrin-mediated endocytosis of virions [86]. In the case of CCHFV, the drug was shown to markedly reduce viral titer when added up to 24-h post infection, suggesting a post entry inhibitory effect [44]. The assembly of mature viral progeny and/or its exit from infected cells might be good candidates, being already identified as the target of action of chlorpromazine in the case of the vesicular stomatitis virus and of the Sindbis virus [67].

The ability to inhibit parvoviral entry has been reported for the antidepressant desipramine by a lipid raft-disrupting mechanism [25]. More recently, Carette and coworkers [87] showed that the psychoactive drug imipramine interferes with the entry of EBOV into target cells. Similar effects have

been reported for different psychoactive drugs, such as the antidepressant drugs sertraline and trimipramine, as well as for the antihistamine/antiemetic drug promethazine [43,52]. Furthermore, sertraline has been reported to inhibit also Zika virus infection [88].

3.4. Selective estrogen receptor modulators (SERMs)

Screening studies for drug repurposing as antivirals showed that SERMs are active against MERS-CoV, EBOV, HCV, and HSV-1 [53–55,57,86]. The anti-EBOV activity of SERMS has been demonstrated even in the absence of detectable expression of estrogen receptor, suggesting that clomiphene and toremifene are not working through classical pathways associated with the estrogen receptor functions [53]. Instead, these compounds would interfere with a late stage of EBOV entry into target cells, likely affecting the triggering of fusion of the viral envelope with the endosomal limiting membrane. In fact, it has been recently shown that toremifene interacts with the EBOV glycoprotein (GP) triggering the premature release of the GP2 subunit, thus preventing the fusion process [89].

Clomiphene and toremifene have been found to affect EBOV infection *in vitro* and in a mouse model [43,53]. Interestingly, clomiphene accumulates in the eye and in the male reproductive tract, where EBOV is known to persist in patients who recovered from the infection [90]. Thus, this compound would potentially act also on EBOV 'reservoirs' in survivor patients [90].

3.5. Protein kinase inhibitors

Several protein kinases are involved in viral trafficking during entry, assembly and release from the infected cells [12]. It has been shown that inhibitors of protein kinases, used as approved anticancer drugs, act *in vitro* against HCV, HIV, several flaviviruses, and EBOV [12]. In particular, the CAD sunitinib inhibits the adapter-associated kinase 1 and cyclin G-associated kinase required for HCV assembly [56]. Sunitinib, in combination with the anticancer drug erlotinib (a protein kinase inhibitor), protects mice from challenges with lethal doses of EBOV and Dengue, while others kinases inhibitors used in the cancer treatment have a broad antiviral activity [43,49,91].

3.6. Miscellaneous cads with antiviral activity

During the last large outbreak of EBOV in West Africa, several papers based on screening studies have been published with the effort to identify molecules with anti-EBOV activity. Among CADs that have been shown to inhibit EBOV infection, there are the antifungal drugs terconazole and triparanol, formerly used cholesterol-lowering drugs, now withdrawn due to their numerous toxic side effects [57].

Among CADs that are still not used in the clinical practice, U18666A deserves some considerations. Indeed, the cholesterol synthesis and transport inhibitor U18666A is widely used as a prototype CAD, in the field of lipid research [23] and its efficacy has been tested against important human relevant pathogens such as, dengue virus, HIV-1, HBV, HCV, Lujo virus, and EBOV [26,57–59,87,92–96] as well as in the development of therapeutic intervention against prion disease [97]. Not only, due to its well-known effects on the cells [23], the U18666A has been employed for the experiments that have led to the identification of the EBOV-intracellular receptor NPC-1 [87,94]. Although U18666A can physically interact with NPC-1, its anti-EBOV activity seems to be independent by such an interaction and more likely due to U18666A pleiotropic effects on the LE/Lys system [23,98].

4. Conclusion

Although CADs are a wide and heterogeneous group of chemicals, they share common chemical-physical characteristics that allow a cellular accumulation into acidic organelles inducing a NPC-like phenotype with alteration of several pathways connected with the endolysosomal compartment.

These cellular alterations affect the viral replication cycle of several viruses, mainly at the entry step but, in same cases, also at the level of the viral assembly/budding.

CADs are effective *in vitro* against a wide number of viruses but limited *in vivo* studies support the *in vitro* observations. However, CADs seem a promising tool to develop broad-range antiviral therapies, in particular to control outbreaks from emerging and reemerging highly pathogenic viruses.

5. Expert commentary

CADs inhibit viral replication with different and unclarified mechanisms and understanding how these molecules interfere with the viral replication cycle could provide the basis for proper evaluation of their therapeutic potential. In this context, modeling studies to evaluate possible interactions between CADs and cellular/viral proteins could shed light on the complex mechanisms underlying CAD antiviral activity. Under this respect, recent studies involving molecular modeling suggest that amiodarone, dronedarone, as well as toremifene might directly interact with the Ebola virus surface glycoprotein [89,99]. On the other hand, at least in the case of amiodarone, studies based on biological assays indicate that the anti EBOV drug activity is not limited to an effect on viral entry but it is also due to effects on the overall cellular physiology, that, in turns, influence viral replication [40,41,53,57]. Taken together, these results further highlight the complexity of the antiviral mechanism of CADs.

Repurposing of clinically approved drugs as antivirals is appealing because good safety data do exist from previous clinical studies, allowing a faster transition into phase II–III clinical trials. In addition, considering that CADs mainly exert their antiviral activity by acting on cellular target, one of their positive aspects is represented by the fact that the development of resistant viral strains is unlikely. Although numerous *in vitro* studies and some *in vivo* evidence in animal models suggest the possibility to employ CADs as antiviral, their use deserves some considerations.

CADs do require a long time to reach tissue plateau concentrations in human tissues if compared to *in vitro* cell culture models [13]. This finding is likely due to the low ratio between the amounts of drug administered and the

total volume of the storage compartment. The physicochemical properties of CADs result in a broad tissue binding, as demonstrated by the high apparent volume of distribution of these drugs in human body districts [13]. Thus, it is mandatory to further analyze in the animal models the efficacy of CADs, in order to determine the appropriate dosage and length of treatment necessary to achieve a stable intracellular concentration that is required for the antiviral activity. Indeed, these pharmacokinetic characteristics, the possibility to have a poor penetration in specific tissues, and differences in viral strains and/or in the genetic background of the studied populations could explain the few *in vivo* confirmations of the antiviral efficiency of CADs. To improve the delivery of CADs, approaches based on nanocarriers, like phospholipid micelles or PEGylated graphene, have been proposed [100,101]. Unfortunately these delivery systems can have drawbacks, such as the delay of CAD release inside the cells, as well as the longer/higher storage of the compounds in the tissues that might increase the risk of CAD side effects and toxicity [101].

Although the CAD typical pharmacokinetic could reduce the efficacy of these molecules as antiviral treatment, they could be used in association with other antiviral compounds, especially in acute setting, as well as in preexposure and/or prophylaxis regimen for people at high risk of infection with highly pathogenic viruses. In this context, it has been recently shown that the combination of more drugs displayed a synergistic effect in inhibiting EBOV replication *in vitro* at reduced concentrations of each single drug [102]. Animal models are also important to carefully evaluate the potential adverse effects that might be associated especially to certain classes of CADs. Indeed, even though several CADs, that have been shown to exert antiviral activity *in vitro*, are already licensed mainly for the treatment of cardiovascular and psychiatric diseases, as estrogen regulators or as antimalarial drugs, the absence of side effects during their off-label use remains a critical aspect that needs to be properly addressed. The problems related to CAD toxicity in human treatment have been already debated during the last EBOV outbreak in West Africa, when a special concern was expressed on the use of amiodarone in clinical trials mainly due to its potential side effects [103,104]. In this context, it has to be mentioned that the treatment of an acute infection with a highly pathogenic virus should require a limited period of CAD administration, thus potentially reducing the risk of side effects. However, this aspect needs to be carefully investigated with appropriate animal models and clinical trials. Finally, despite CADs display a wide range of antiviral effects, surprisingly some reports described a positive effect of specific CADs on replication of certain viruses. For instance, Salata et al. [66] reported that amiodarone significantly increases enteroviral progeny release from infected cells *in vitro*, while Wu et al. [105] described an increase of influenza A virus A/WSN/33 (H1N1) replication in human lung epithelial cells A549, after treatment with chloroquine. Klintworth and coworkers [65] reported that, while amiodarone and other CADs do not affect the entry of wild type rabies virus into target cells, the same molecules enhance entry of lentiviral particles pseudotyped with the rabies virus envelope glycoprotein into non-neuronal cells. Of note, Klintworth's study also highlights the fact that, although pseudo-viruses are good surrogates to screen viral entry inhibitors for pathogens requiring high containment level laboratories, data based on this tool need to be validated by performing experiments with the corresponding wild-type pathogenic virus. Overall, these reports strongly suggest that a careful monitoring of CAD effects on the load of viruses that could infect/coinfect individuals treated with these compounds, both for labeled as well as for off-label applications, should be considered.

6. Five-year view

The recent emergence and reemergence of different highly pathogenic viruses around the world makes the development of effective antivirals one of the top Public Health priorities.

Repurposing approved drugs for treating emerging infections is a potential resource for rapidly setting up therapeutic responses toward a broad spectrum of pathogens. CADs represent interesting candidates for drug repurposing and further studies are requested to prove their real effectiveness in the field of the infectious diseases treatment. In addition, CADs can also provide a rational platform to develop new derivatives optimized to achieve a strong suppression of viral replication with less side effects with respect to their parental compounds.

Thus, we foresee that this family of pharmaceuticals has a high potential, especially against highly pathogenic viruses, and it is worth to be further investigated also in view of viruses that might emerge as public health threat in the near future.

Finally, CADs will continue to represent a useful tool in lipid research and could find a role also in the dissection of the complex interplay between the host cell and viruses.

Key issues

- An increasing number of outbreaks due to wide array of emerging and re-emerging highly pathogenic viruses was observed since year 2000.
- No specific drugs are still available for most of the emerging/re-emerging highly pathogenic viruses, then there is an urgent need for new drugs.
- New approaches are focalized on the identification of broad-spectrum antivirals targeting characteristics of the viral life cycle shared by different viruses.
- Cationic amphiphilic drugs (CADs) interact with different cellular membranes and accumulate in acidic intracellular compartments such as late endosomes/lysosomes, thus affecting cellular pathways/structures required for the replication of several viruses.
- Several FDA-approved drugs are CADs and can be studied in drug repurposing screening to discovery new application as antivirals.
- CADs can also represent tools to study viral replication.

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Declaration of interest

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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