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REVIEW ARTICLE

Development of antiviral agents toward enterovirus 71 infection

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Enterovirus 71 (EV71) infection remains a public health problem at a global level, particularly in the Asia-Pacific region. The infection normally manifests as hand–foot–mouth disease; however, it is capable of developing into potentially fatal neurological complications. There is currently no approved vaccine or antiviral substance available for the prevention or treatment of EV71 infection. This paper, thus, reviews efforts to develop or discover synthetic as well as naturally occurring compounds directed against EV71 infection. The recent achievements in cellular receptors of EV71 are also highlighted, and their contribution to the development of antiviral drugs against EV71 is discussed in this article.

Introduction

Enterovirus 71 (EV71) is a pathogenic serotype of the Picornaviridae family, containing a single positive sense RNA genome with a length of approximately 7.5 kb. Detected during a small outbreak in California between 1969 and 1972,[1] EV71 has now been turned into one of the most pathogenic Enterovirus serotypes with many outbreaks occurring around the world, particularly in the Asia-Pacific region. Although EV71 infection usually manifests with rashes and vesicular lesions on the hands, feet, and oral mucosa,[2] it sometimes leads to fatal neurological complications such as aseptic meningitis, encephalitis, acute respiratory disease, and pulmonary edema.[3] Following the large outbreaks in both Malaysia and Taiwan in the late 1990s,[4] new life-threatening outbreaks of EV71 reoccurred in Taiwan in 2001–2002[5] and in China in 2010.[6]

There is no approved vaccine or antiviral drug available for prophylaxis or treatment of EV71 infection to date.[7] Therefore, research studies should continue to develop

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novel EV71 inhibitors and as such, latest achievements in this field always have to be updated. At least three recent reviews have detailed a range of studies undertaken in both fields of EV71: antivirals and vaccines.[7–9] However, compared to EV71 vaccine development, a large number of research ventures conducted into EV71 antiviral development has generated vast information on novel compounds with anti-EV71 capacity and even improved the understanding of EV71–host interactions. Hence, this review attempts to provide a focused body of information regarding the status and challenges of antiviral therapy for EV71, covering both options: synthetic and natural bioactive compounds. In addition, we discuss how newly discovered EV71 cellular receptors might lead to new avenues for anti-EV71 drug design.

EV71 structural protein inhibitors

It is well known that variations within capsid proteins VP1–VP3 of EV71 are responsible for the antigenic diversity of the virus, whereas neutralization sites are most densely clustered on VP1.[10] Thus, EV71 VP1 inhibitors have been suggested as one of the first candidates for developing antivirals against the viral infection. Thus far, pleconaril has been known as a viral capsid binder that has exhibited inhibitory effects against a number of Enteroviruses[11,12] except EV71.[10] However, a recent study interestingly showed that pleconaril could significantly increase the viability of EV71-infected cells and reduce the mortality of EV71-infected mice.[13] Therefore, it remains to be investigated whether pleconaril should be considered a potent EV71 inhibitor.

Among other capsid binders, pyridyl imidazolidinone was the first to demonstrate notable potencies against EV71 infection in a number of consecutive studies.[10,14–17] Pyridyl imidazolidinone is a new category of capsid binders, generated by a computer-assisted drug design.[10] It is believed that pyridyl imidazolidinone exerts its antiviral action by fitting into the viral hydrophobic pocket of VP1.[17] In the pyridyl imidazolidinone family, BPROZ-101 with a half-maximal inhibitory concentration (IC_{50}) of 0.0012 ± 0.0005 (μM) and BPROZ-074 with an IC_{50} of 0.0008 ± 0.0001 (μM) exhibited the most considerable antiviral activities against EV71.[17] In addition to pyridyl imidazolidinone, an EV71 VP1-derived peptide was also demonstrated to reduce the cytopathic effects of all EV71 strains from genotypes A, B, and C, with IC_{50} values ranging from 6 mM to 9.3 mM in RD cells.[18]

EV71 nonstructural protein inhibitors

The seven nonstructural proteins of EV71 are the essential elements involved in various viral functions including proteases, RNA replication, ATPase activity, RNA helicase activity, and RNA replication. Hence, these peptides have been studied as potential targets for designing antiviral agents toward EV71.[19]

$2A^{pro}$

Recently, it was shown that a six-amino-acid peptide, LVLQTM, exhibited antiviral potencies against EV71 in HeLa cells. This peptide was shown to serve as a substrate mimetic of the EV71 $2A^{pro}$ that is known to be responsible for the viral protease activity.[20]

2B

Little information is available for the function of the 2B peptide of EV71, but it is thought to be important for EV71 RNA replication.[21] In this regard, 4,4′-diisothiocyano-2,2′-stilbenedisulfonic acid was shown to prevent EV71 2B activity, leading to the inhibition of virus production in RD cells.[22]

2C

As a multifunctional protein, EV71 2C has been suggested to be involved in processing nucleoside triphosphatase activity[23] and synthesis of RNA negative strands.[24] Two adenosine analogues—Metrifudil and N^6-benzyladenosine—have been demonstrated to interact with the EV71 2C peptide and inhibit viral infection.[25]

3A

EV71 3A is highly conserved and serves as a scaffold of the viral RNA replication complex.[19] In this respect, an enviroxime mimetic compound, AN-12-H5, was reported to target the 3A region of EV71 and significantly inhibit an early stage of EV71 infection after virus binding.[26]

$3C^{pro}$

The EV71 3C peptide acts as a viral protease during the viral infection of host cells, playing a vital role in the maturation of virion particles. A study showed that rupintrivir promisingly inhibited EV71 3C by mimicking the substrate of the 3C protease.[27] The anti-EV71 activity of rupintrivir was also evaluated *in vivo*,[28] where the rupintrivir-treated suckling mice were largely protected from EV71-caused limb paralysis. Because of its safety, it has been concluded that rupintrivir can be suitable for immediate evaluation as a therapy in EV71-infected individuals with fatal neurological complications.[28]

$3D^{pol}$

EV71 $3D^{pol}$ is an RNA-dependent RNA polymerase that serves as a key element for the viral RNA replication process. DTriP-22 as a nonnucleoside analogue was shown to inhibit EV71 infection by reducing the viral RNA accumulation after virus absorption.[29] In addition to DTrip-22, aurintricarboxylic acid (a polyanionic compound) could prevent EV71 infection through interference with $3D^{pol}$ in Vero cells.[30]

Nucleotide analogues

Ribavirin (RBV) in the form of ribavirin triphosphate can serve as a base analogue of either ATP or GTP. The antiviral potency of RBV has been presented for a wide range of RNA viruses *in vitro* and/or *in vivo*,[31–39] including EV71[40] where