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Cyclophilin A Inhibitor: Potential Therapeutics for the Treatment of COVID-19

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Abstract

In December 2019, an outbreak of pneumonia caused by a new Coronavirus (SARS-CoV-2) occurred and spread rapidly throughout the world. There were other severe coronavirus outbreaks worldwide, namely severe acute respiratory syndrome (SARS-CoV) and MERS-CoV. Because the genetic diversity of coronavirus complicates the design of vaccines, broad-spectrum anti-coronavirus drugs have become an important approach to control the coronavirus epidemic. Cyclophilin A is an important protein needed for coronavirus replication and its inhibitor cyclosporine has the ability to suppress coronavirus on a broad spectrum. However, cyclosporine has an immunosuppressant function, so the conditions for its use as an antiviral drug are limited. As a result, cyclosporine A analogues without immunosuppressive side effects have attracted lots of interest. This review primarily discusses the drug development prospects of Cyclophilin A as a drug target for the treatment of coronavirus infection, especially (Coronavirus disease 2019) COVID-19, and non-immunosuppressive cyclosporine analogues.

Keywords: Cyclophilin A; Cyclosporine A; COVID-2019; SARS-CoV2

Introduction

Coronavirus (CoV) is the general name of a family of viruses that is commonly found in nature [1]. These viruses are able to utilize a wide variety of host species, and because host switching is a common feature of CoV evolution, novel CoVs may appear at any time [2]. Currently, seven different Coronaviruses (SARS-CoV, hCoV-NL63, hCoV-HKU-1, hCoV-OC43, hCoV-229E, MERS-CoV, and SARS-CoV-2) are currently reported to cause respiratory diseases in humans [2-4]. The December 2019 outbreak of a new coronavirus in China's Wuhan region, recently named SARS-CoV-2 and "COVID-19" was announced as the name of this new disease by the World Health Organization (WHO), belongs to the coronavirus 2B type, which is about 80% similar to the SARS-CoV structure. There is still the possibility of continued mutation in the future [5,6]. A variety of treatments for CoVs are being developed, including immunomodulation, vaccination, CoV-specific Direct-Acting Antivirals (DAAs), and host-specific antivirals.

Cyclophilins (Cyps) are a commonly expressed and highly conservative family of intracellular proteins that have activity of Peptidyl-Prolylcis-trans Isomerase (PPIase). Cell receptors originally found as immunosuppressive drug Cyclosporine A (CsA) were found [7,8]. At this stage, CsA can suppress the Coronavirus, which has raised wide spread scientific research interest. This paper analyzes the development prospects and potential of Cyclophilin inhibitors as drug store at coronaviruses, especially SARS-CoV-2, and provides reference for future drug research and development.

Introduction of coronaviruses

Coronaviruses are globular viruses with a membrane, as well as age home of about 26 kb to 32 kb of non-fragmental justice single-stranded RNA that contains seven to ten different Open Reading Frames (ORF) [9,10]. Coronaviruses are enveloped positive-sense RNA viruses, and has 26 known types, including alpha, beta, and radon, based on sequence comparisons of entire viral genomes. There are a total of four genera, of which only alpha and beta are disease-causing in humans [11].

Coronaviruses contain at least four major structural proteins, including Spike (S), Membrane (M), Envelope (E), and Nucleocapsid (N) proteins, all encoded at 3'end ORF in the genome. Functionally, S glycoproteins are involved as the main antigens in receptor bind in and cell fusion [9], while M proteins are primarily involved in germination and envelope formation, and play

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an important role in subsequent viral particle assembly [12]. These structural proteins are essential for viral-cell receptor binding [13,14]. Coronaviruses can cause respiratory and intestinal infections in animals and humans [15].

According to sequencing results, the gene sequence similarity between the SARS-CoV-2 virus and SARS-CoV was 75% to 80% [16,17]. It is speculated that its animal host may be a bat, but SARS-CoV may also exist as an intermediate host [18]. Highly pathogenic Coronavirus has been endemic three times in the past 20 years. Two highly transmitted and pathogenic diseases, including SARS-CoV and MERS-CoV, have previously becomes ever respiratory epidemics worldwide [19,20].

At present, there is no specific treatment for coronavirus, and there is no effective treatment plan for severely ill patients. In deciding how to treat coronavirus infection, especially the recent COVID-19 infection, it is important to find viable therapeutic targets and effective therapeutic drugs. At present, it has been found that Cyclophilin inhibitors can inhibit coronavirus replication, providing a basis for the targeted development of broad-spectrum coronavirus inhibitors.

Cyclophilin inhibitors

Introduction of cyclophilin: Cyclophilins (Cyps) are a commonly expressed and highly conservative class of intracellular proteins belonging to family of PPIase [21,22]. Cyps play a very important role in the replication of RNA viruses (including influenza A viruses, HIV, HCV, and others). Cyclophilins are known to be present in the cells of eukaryotes and prokaryote organisms [22]. There are seven main types of Cyclophilins in the human body, namely Cyclophilin A (CyPA), Cyclophilin B (CypB), Cyclophilin C (CypC), Cyclophilin D (CypD), Cyclophilin E (CypE), Cyclophilin 40 (Cyp40), and Cyclophilin NK (Cypp). They are not typically connected to each other in the genome [23]. Of these, CyPA is the most abundant and dominant protein in the Cyps family. CyPA plays an important role in intracellular protein synthesis, folding, and transportation, as well as immunosuppression, immunomodulation, and signal conduction. However, CyPA are closely related to viral infections such as Human Immunodeficiency Virus (HIV), Hepatitis B Virus (HBV) and Hepatitis C Virus (HCV), Rheumatoid arthritis, asthma, Alzheimer's disease, and cardiovascular disease [7,8]. In addition, CyPA is associated with hepatitis and fibrosis caused by viral infections such as HBV, HCV and non-alcoholic fatty liver, as well as the generation of resistance to liver cancer and liver cancer metastasis, making it a potential target for the treatment of liver disease [8].

Cyclosporine A and cyclophilin: CsA was originally discovered by S and Ersin the process of screening non-cytotoxic immunosuppressants, and it was developed for using in organ transplantation [24,25]. In depth study found that its immunosuppressive effect is due to the formation of two sub-prime complexes with Cyps and Calcineurin (CaN), which protect the transcription of activated T-cells (NFAT).

CaN can participate in the transcription process of some cytokines such as Interleukin-2 (IL-2) by dephosphorylation and activation of NFAT. Therefore, the interaction between CsA and Cyps can inhibit the release of immune-related cytokines, thus exerting the immunosuppressive effect [25-29].

CsA is a ligand that can be combined with CyPA. When combined, the molecular structure of CsA changes significantly compared with its unbound structure. The original hydrogen bonds in the molecules are broken, and a new hydrogen bond is formed [30]. In addition, proline-containing peptides are converted from cisto-trans, so that the resulting compound can be combined with CaN, while neither CsA nor CyPA alone can bind to CaN. CsA, with a molecular size of 11 amino acid cyclopeptides, has a surface bound to CyPA's PPI as trench, as well as a surface that can bond with CaN.

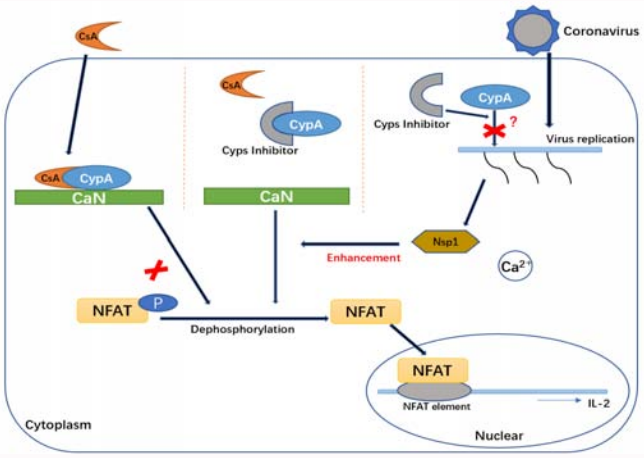


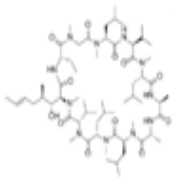
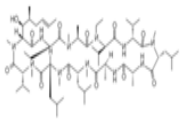
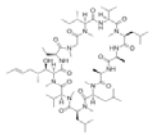
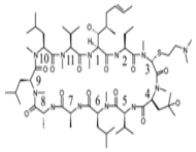
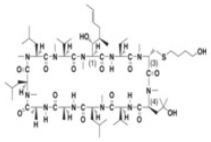
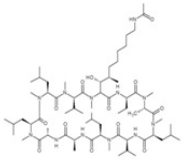
Figure 1: The role that CyPA plays in the NFAT pathway and replication of Coronavirus. CsA-CypA complex can inhibit the Phosphorylation activity of CaN. CaN participate in the transcription process of some cytokines such as IL-2 by Dephosphorylation of NFAT. Nsp1 protein can enhance NFAT activities with $Ca2^{+}$. However, little is known about the exact role of CypA in CoV replication.
CaN: Calcineurin; CsA: Cyclosporin A; CyPA: Cyclophilin A; Nsp1: Non-structure protein 1; IL-2: Interleukin-2; NFAT: Nuclear Factor of Activated T-cells

Study of cyclophilin as a therapeutic target for coronavirus: CyPA plays an extremely important role in HCV, Human Papillomavirus (HPV), HIV-1 and bovine pox virus [31]. As a ligand of CyPA, it has been found that CsA can prevent the replication of most CoVs by inhibiting Cyclophilin proteins, including SARS-CoV, hCoV-229E, and hCoV-NL63, as well as the avian infectious bronchitis virus [4,31-35].

CyPA is a potential drug target for Coronaviruses [33]. Brunn's team at LMU University in Germany reported that the nucleocapsid protein of SARS-CoV binds closely to CyPA in humans, and that inhibiting the binding of two proteins by CsA inhibits the replication of hCoV-229E [4,35,36]. In 2011, it was reported that CsA inhibits CoV infection [32]. In the same year, the Brunn team demonstrated that the host's Cyps were a possible target for CoV, and also noted that non-immunosuppressive cyclosporine derivatives may have the potential to become a broad-spectrum CoV inhibitor [33]. Later, in 2014, the Brunn team further confirmed that the low-host CyPA could prevent the replication of hCoV-NL63.

In vitro, CsA can inhibit the replication of almost all coronavirus generaina dose-dependent manner [33,37]. Cell culture experiments have found that CsA can strongly inhibit the replication of SARS-CoV, MERS-CoV, hCoV-229E, poultry infectious bronchitis virus, and mouse hepatitis virus [33], but only showed significant blocking in the early stages of replication [32]. Blocking Coronavirus replication requires a higher CsA concentration (16 μM) than other RNA viruses (0.5 to 3 μM), indicating that the CoV is less sensitive to CsA treatment [32]. As an effective and broad spectrum CoV

Table 1: Structure and current research status of some Cyclophilin inhibitors.

Name	Structure	Corporation	Indication	Activity	Clinical trial stage
CsA		Norvatis	Indicated for treatment of adult, non-immunocompromised patients with severe, recalcitrant, plaque psoriasis who have failed to respond to at least 1 systemic therapy (eg. PUVA, retinoids, or methotrexate) or in patients for whom other systemic therapies are contraindicated, or cannot be tolerated	CsA inhibits the replication of diverse coronaviruses at non-cytotoxic, low-micromolar concentrations. Treatment of infected cells with 16 μM CsA strongly reduced viral and reporter gene expression of SARS-CoV–GFP, the amount of dsRNA in infected cells and the virus titre in culture supernatants (by >3 logs) [33].	Indication mentioned previously: **On market** Hepatitis C Viral Infection: **Phase IV completed**
Alisporivir		Norvatis	Hepatitis C; Chronic Hepatitis C	In cell culture models, low-micromolar doses of alisporivir block SARS-CoV and MERS-CoV replication. Combination treatment with alisporivir and ribavirin increases the anti-MERS-CoV activity in cell culture [41].	Gastrointestinal disorders: **preclinical** Chronic Hepatitis C Virus Infection/Hepatitis C Viral Infection: **Phase III completed**
NIM811		Novartis	Hepatitis C; HIV-1 infection	Induces a concentration-dependent reduction of HCV RNA in the replicon cells with an IC50 of 0.66 μM at 48h [42].	Chronic Hepatitis C Genotype-1 Relapse: **Phase II completed**
SCY-635		SCYNEXIS, Inc.	Hepatitis C; Hepatitis B	The EC50 is determined by using the luciferase end point following incubation for 24, 48, 72, and 120h were 0.20, 0.07, 0.08, and 0.15 μM, respectively in the HCV subgenomic replicon system [43].	Hepatitis C: **Phase II** Hepatitis B: **No development reported**
STG-175		S & T Global	Hepatitis B	We found that STG-175 inhibits at a nM range the replication of all HCV genotypes: GT1a (EC50=13.5 nM), GT1b (EC50=15.1 nM), GT2a (EC50=11.5 nM), GT3a (EC50=38.9 nM) and GT4a (EC50=15.2 nM)[44].	Hepatitis B: **Preclinical**
CPI-431-32		Ciclofilin Pharmaceuticals Inc	Hepatitis B	Effectively inhibits HIV-1/HCV co-infection; shows efficacy against drug-resistant HIV-1 and HCV variants. A daily CPI-431-32 dose of 0.5 μM is used. The repeated addition of CPI-431-32 almost totally repressed HIV-1 replication after fifteen days of drug treatment [45].	Hepatitis B; Neurological disorders; Reperfusion injury: **Preclinical-discontinued** HIV-1, HCV: **Preclinical** A Study in Healthy Volunteers and Patients With Chronic Hepatitis B: **Phase I-Active, not recruiting**
CRV431		Hepion Pharmaceuticals	Hepatitis B	In transgenic mouse, the low dose of CRV431 (10 mg/kg/day) reduced HBV DNA levels by only 13% compared to the vehicle group, the higher dose (50 mg/kg) reduced the mean HBV DNA level by 91%. Serum CRV431 drug levels at 3 hr post-dose on the final day of dosing were 0.01 μg/ml (± 0.01 μg/ml SD) and 0.34 μg/ml (0.22 μg/ml SD) in the low-dose and high-dose groups, respectively [46].	Hepatitis B: **Phase I/II** Hepatitis D; Liver cancer; Non-alcoholic fatty liver disease; Non-alcoholic steatohepatitis: **Preclinical** Coronavirus infections; Hepatitis C; HIV-1 infections: **Discontinued**

inhibitor, CsA and its analogues have good research and development prospects.

Prospects for non-immunosuppressive cyclophilin inhibitors: As an immunosuppressive drug, CsA has a broad spectrum of antiviral effects [8,36], but its immunosuppressive properties can lead to adverse side effects during antiviral therapy. As a result, several non-immunosuppressive Cyclophilin inhibitors based on CsA structures are being developed, and these non-immunosuppressive CyPA inhibitors separate their function of PPIase inhibition with immunosuppressive function (Table 1). The side chains of the modified CsA molecule can develop non-immunosuppressive analogues such as NIM811, Alisporivir (Debio-025), SCY-635, sangliferins, and STG-175. In order to better understand the structural diversity of CyPA inhibitors, more non-immunosuppressive CsA derivatives need to be developed. Known co-crystal structures provide the basis for drug optimization of non-immunosuppressive Cyclophilin inhibitors.

It was reported that multiple derivatives of non-immunosuppressive CsA were effective in inhibiting h CoV-229E

replication, indicating that they could be a candidate drug for the treatment of CoV infection in humans [4,36]. CsA and its derivatives interrupt the interaction between CyPA and CaN proteins [38]. The study also found that in cells, CyPA inhibitors-alisporivir can inhibit the replication of four different Coronaviruses (including MERS and SARS CoVs), and in cell-based infection models, ribavirin further enhances alisporivir's antiviral effect [39-44].

Conclusions and Prospects

In the past 20 years, from SARS-CoV in 2002, MERS-CoV in 2009, to the recent SARS-CoV-2 epidemic, there are three outbreaks of severe infectious diseases caused by CoVs, which requires high attention. In recent years, the results of genomic sequencing and bio informatics for these CoV shave enabled the creation of screening models, which have been used to develop small molecule drugs and vaccines with different mechanisms of action, such as protease inhibitors, nucleic acid synthesis inhibitors and polymerase inhibitors. However, there have been no potent and specific drugs developed for Coronaviruses.

As an important intracellular protein in RNA viruses, Cyps have been shown to play an important role in virus replication. In addition, CyPA is considered to be a potential drug target for CoVs, and its inhibitory ligand CsA has been shown to inhibit the replication process of a variety of CoVs. However, as an immunosuppressant inhibitor, the side effects caused by CsA in clinical applications cannot be ignored. The development of non-immunosuppressive inhibitors based on CsA's structure has begun to attract attention.

A variety of non-immunosuppressive Cyclophilin inhibitors are currently being developed, such as alisporivir, SCY-635, NIM811, and CRV431. CyPA target affinity for these inhibitors has been extensively validated in HCV. Furthermore, some studies have found that many of these inhibitors can effectively inhibit the replication of hCoV-229E, indicating its potential as a treatment for human CoV infection.

There is still no complete validation of the inhibitory effect of non-immunosuppressive Cyclophilin inhibitors and therapies on Coronaviruses, especially COVID-19. However, as a highly promising class of drugs, non-immunosuppressive CyPA inhibitors will attract more attention.

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