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COMMENTARY

Plitidepsin: a Repurposed Drug for the Treatment of COVID-19

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ABSTRACT Finding antivirals to reduce coronavirus disease 2019 (COVID-19) morbidity and mortality has been challenging. Large randomized clinical trials that aimed to test four repurposed drugs, hydroxychloroquine, lopinavir-ritonavir, interferon beta 1a, and remdesivir, have shown that these compounds lack an impact on the COVID-19 course. Although the phase III COVID-19 vaccine trial results are encouraging, the search for effective COVID-19 therapeutics should not stop. Recently, plitidepsin (aplidin) demonstrated highly effective preclinical activity against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Its antiviral activity was 27.5-fold more potent than that of remdesivir (K. M. White, R. Rosales, S. Yildiz, T. Kehrer, et al., Science, 2021, https://science.sciencemag.org/content/early/2021/01/22/science.abf4058). Plitidepsin, a repurposed drug developed for the treatment of multiple myeloma, targets the host translation cofactor eEF1A. Plitidepsin has shown efficacy in animal models and phase I/II human trials. Although plitidepsin is administered intravenously and its toxicity profile remains to be fully characterized, this compound may be a promising alternative COVID-19 therapeutic.

KEYWORDS COVID-19, plitidepsin, experimental therapeutics

By the end of January 2021, over 100 million people were infected with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The magnitude of the CoV disease 2019 (COVID-19) pandemic has promoted the repurposing of several drugs, with the aim of rapidly stopping the morbidity, mortality, and spread of this new disease. Repurposed antiviral drugs tested to fight COVID-19 have been chosen, mainly based on promising *ex vivo* efficacy against SARS-CoV-2 or based on previous therapeutic results with other human coronavirus diseases, such as SARS-CoV and Middle East respiratory syndrome CoV (MERS-CoV) (1). Numerous clinical trials have been completed, but no repurposed antiviral drug has been found that could significantly impact the course of the COVID-19 pandemic (2, 3). COVID-19 vaccines have recently been released by several manufacturers for protection against severe disease (4). Current COVID-19 vaccines are being administered intramuscularly or intradermally, and they will induce mostly immunoglobulin G (IgG) production, but no secretory IgA production (5). Consequently, these vaccines can probably prevent disease but will probably not generate sterilizing immunity; that is, they may allow transmission of the virus (5). Another concern is the potential magnitude and durability of the vaccine-induced responses. In this scenario, addressing the current pandemic will require different strategies utilized in concert, including effective and competent antivirals.

A recent (2021) report by White et al. showed that plitidepsin (aplidin) had potent antiviral activity against SARS-CoV-2 (6). Plitidepsin inhibited SARS-CoV-2 in Vero E6 cells, with a 50% inhibitory concentration (IC_{50}) of 0.70 nM and with a 50% cytotoxic concentration (CC_{50}) of 1.99 nM. Next, the potency of plitidepsin was tested in the human cell line hACE2-293T (IC_{50} = 0.73, CC_{50} > 200 nM) and in pneumocyte-like cells (IC_{50} = 1.62, CC_{50} = 65.43 nM). These results demonstrated the *ex vivo* potency of plitidepsin, although they also revealed the cell toxicity of this compound. In parallel, in

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the same tissue culture cells, the potency of remdesivir was tested. The results showed that plitidepsin was 27.5-fold more potent than remdesivir, although it was also more cytotoxic (CC_{50} = 1.99 to 200 nM versus 2 to 20 μM). Remarkably, remdesivir is the prodrug of an adenosine analogue which was approved by the Food and Drug Administration for treating hospitalized patients with COVID-19 (7). The rationale for testing plitidepsin against SARS-CoV-2 was that this compound was thought to target the host eEF1A protein (8). eEF1A is responsible for the enzymatic delivery of aminoacyl tRNAs to the ribosome during eukaryotic protein translation. It was previously identified as one of the 332 host proteins implicated in the life cycle of SARS-CoV-2 (9). In their recent study, White et al. elegantly demonstrated that plitidepsin targeted eEF1A. They engineered cells that expressed a mutated version of this protein (eEF1A-A399v) (6) and confirmed that eEF1A was a druggable target for inhibiting SARS-CoV-2 replication.

The *in vivo* efficacy of plitidepsin was then tested in two animal models of the SARS-CoV-2 infection. First, wild-type BALB/c mice were transduced with human ACE2 and prophylactically treated either with 0.3 mg plitidepsin/kg of body weight per day for 2 days or with a single dose of 1-mg/kg plitidepsin. After 2 h of treatment, mice were infected with 1×10^4 PFU of SARS-CoV-2. On day 3 postinfection, a 2-log reduction in SARS-CoV-2 viral titers was observed in the lungs of the 0.3-mg/kg plitidepsin group, compared to the viral titer in the vehicle control group. Additionally, a 1.5-log reduction in viral titers was observed in the single-dose 1-mg/kg plitidepsin group. Similar results were observed with a very high concentration of remdesivir (50 mg/kg). After the same protocol was carried out with the K18-hACE2 mouse model, which supported a robust SARS-CoV-2 infection, almost identical results were achieved. Of note, at 3 days postinfection, histopathology showed a reduction in lung inflammation in plitidepsin-treated mice compared to that in control and remdesivir-treated mice (6). Remarkably, a slight loss of body weight in mice treated with daily plitidepsin suggested that the drug might be toxic.

Plitidepsin is a compound extracted from the ascidian *Aplidium albicans*; it belongs to the Pharmamar Company, where it was first clinically developed for the treatment of multiple myeloma (10). Plitidepsin appears to lead to cell cycle arrest, growth inhibition, and apoptosis induction via multiple pathway alterations, which may explain its observed toxicity. A phase III randomized trial in patients with relapsed/refractory multiple myeloma reported outcomes for plitidepsin plus dexamethasone compared to dexamethasone alone. The median progression-free survival was 3.8 months in the plitidepsin arm and 1.9 months in the dexamethasone arm (8). Importantly, dexamethasone, a steroid that does not directly inhibit viral replication, is the only compound that has shown convincing evidence of reducing COVID-19 mortality (11). Because the safety profile of plitidepsin with dexamethasone is known, a trial of these two compounds for treating COVID-19 should be guaranteed. Plitidepsin has also completed a phase I/II clinical study for the treatment of COVID-19 (Spanish Clinical Trials Registry no. 2020-001993-31 [http://reec.aemps.es/reec/public/detail.html]; ClinicalTrials.gov no. NCT04382066 [https://clinicaltrials.gov/ct2/show/NCT04382066?term=plitidepsin&draw=2&rank=8]). Although the only released data from that study were from the Pharmamar Company, they suggested promising results. In that study, three patient cohorts that required hospital admission for COVID-19 were treated with three different plitidepsin dose levels (1.5 mg, 2.0 mg, and 2.5 mg), administered over three consecutive days. The study disclosed a substantial reduction of the viral load in patients between days 4 and 7 from the start of treatment. On day 7, the average viral load was reduced by 50%, and on day 15, it was reduced by 70%. Finally, 80.7% of patients were discharged on or before the 15th day of hospitalization, and 38.2% were discharged before the 8th day (according to the protocol, the minimum required hospitalization was 7 days).

White et al. noted that eEF1A had previously been described as an important host factor for the replication of many viruses, including influenza and respiratory syncytial

viruses, and it was detected in SARS-CoV virions (6). Consequently, they argued that eEF1A inhibition could be a strategy for treating other human coronaviruses and unrelated viruses. For instance, they also showed that plitidepsin retained its efficacy against the new SARS-CoV-2 B.1.1.7 variant (12). Nevertheless, some limitations must be addressed. Preclinical studies must be performed to search for the SARS-CoV-2 protein that interacts with the host eEF1A to confirm the virus specificity of plitidepsin. The generation of SARS-CoV-2 variants in tissue culture that are resistant to plitidepsin should also shed light on its mechanism of action. Like remdesivir, plitidepsin has to be administered intravenously, which might strongly impact its broad utility, particularly as a prophylactic compound. In future, plitidepsin analogues that can be taken orally might be of great utility, considering that COVID-19 is not going to be the last coronavirus or human virus pandemic. Lastly, our experience with other viruses has taught us that targeting host cell cofactors is rather complicated and associated with important toxicity problems. Successful antiviral stories are associated with drugs that target viral proteins. However, the results obtained so far with plitidepsin merit further studies and trials.

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I declare no conflict of interest.

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