Plitidepsin is a Broad-Spectrum Antiviral Shaping the Cell Proteostatic Balance

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Daniel Perez-Zsolt[1,*], Elisa Molina Molina[1,*], Joan Josep Bech-Serra[2], Roger Badia[1,3], Eva Riveira-Muñoz[1], Martin Sachse[4], Sandra Franco[1], Pablo Avilés[5], Miguel Ángel Martínez[1], Roger Paredes[1,6], Bonaventura Clotet[1,3,6,7,8], Cristina Risco[4], Ester Ballana[1,3], Carolina de la Torre[2] and Nuria Izquierdo-Useros[1,3,6] on behalf of the *Broad-Spectrum Antivirals Study Group*

[1]IrsiCaixa AIDS Research Institute, Badalona, Spain, [2]International Josep Carreras Leukaemia Research Institute (IJC), Badalona, Spain, [3]Germans Trias i Pujol Research Institute (IGTP), Badalona, Spain, [4]Centro Nacional de Biotecnología (CNB), Madrid, Spain, [5]PharmaMar S.A., Colmenar Viejo, Madrid, Spain, [6]Consorcio Centro de Investigación Biomédica en Red de Enfermedades Infecciosas (CIBERINFEC), Instituto de Salud Carlos III, Madrid, Spain, [7]University of Vic-Central University of Catalonia (UVic-UCC), Vic, Spain, [8]Fundació Lluita contra la SIDA, Infectious Diseases Department, Hospital Germans Trias i Pujol, Badalona, Spain; *Equal Contribution.

BACKGROUND

Different viruses employ similar pathways for replication, revealing key intracellular hotspots to target with host-directed therapies and achieve a broad-spectrum antiviral activity. Plitidepsin is a clinically approved antitumoral agent that blocks the elongation factor eEF1A required for protein translation. This drug counteracts SARS-CoV-2 replication and shows a favorable safety profile in COVID-19 patients. Yet, the precise antiviral mechanism of action of plitidepsin remains unknown.

Here, we sought to decipher the **mechanism of action** of **plitidepsin** against **SARS-CoV-2** and identified its potential against **other viruses**.

METHODS

We used a deep quantitative proteomic analysis to measure the impact of plitidepsin on the proteome of SARS-CoV-2-infected Vero E6 cells. This was complemented with transmission electron microscopy assays, which unraveled the subcellular and morphological changes associated to plitidepsin treatment. In addition, we performed functional *in vitro* assays to dissect the antiviral activity of plitidepsin against other viruses aside from SARS-CoV-2.

RESULTS

We found that plitidepsin inhibited the synthesis of all SARS-CoV-2 proteins (**Fig. 1A**). These included the R1AB polyproteins (**Fig. 1A**), which facilitate the synthesis of non-structural proteins involved in the formation of double membrane vesicles (DMV). As these subcellular structures are required for viral replication, we observed that plitidepsin reduced DMV formation and the morphogenesis of new viruses (**Fig. 1B**).

Plitidepsin had a greater impact on viral than on host proteins, with less than 14% of the cellular proteome being significantly affected by the drug (**Fig. 2A**). Of note, plitidepsin induced the up-regulation of key molecules associated with protein biosynthesis (**Fig. 2B**), such as the translation initiation factors eIF4A2 and eIF2S3 (**Fig. 2C**). Therefore, plitidepsin induced a compensatory state that rescued protein translation. This proteostatic response explains how cells preserve the cellular proteome after treatment with a translation inhibitor.

Hence, it suggests that plitidepsin could inhibit other RNA-dependent and non-integrated DNA viruses, as we confirmed *in vitro* using Zika virus, Hepatitis C virus replicon and Herpes simplex virus (**Fig. 3A**).

Deciphering the mechanism of action of **plitidepsin** against SARS-CoV-2 revealed its **broad-spectrum antiviral** profile

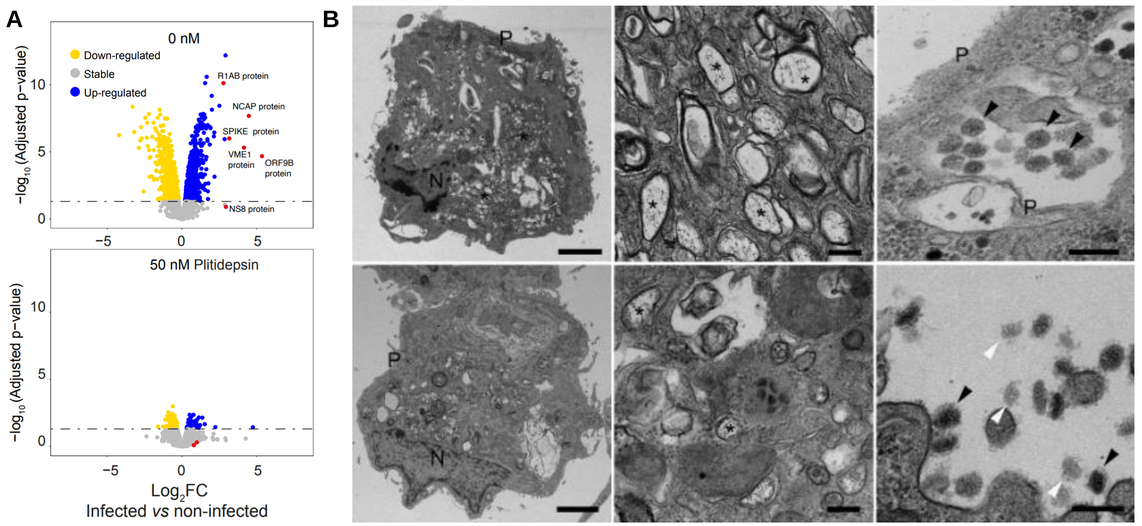

***Fig. 1. Plitidepsin blocks the synthesis of SARS-CoV-2 proteins and the formation of viral particles**. **A**. Changes in cell and viral proteome in mock- and plitidepsin-treated cells (infected vs. non-infected). FC: fold-change. **B**. Transmission electron microscopy of mock- (upper panels) and plitidepsin-treated (lower panels) cells. P: plasma membrane; N: nucleus; *: DMV; black arrows: normal viral particles; white arrows: misshapen viral particles. Scale bars: 2μm (left panels) and 200nm (rest of panels).*

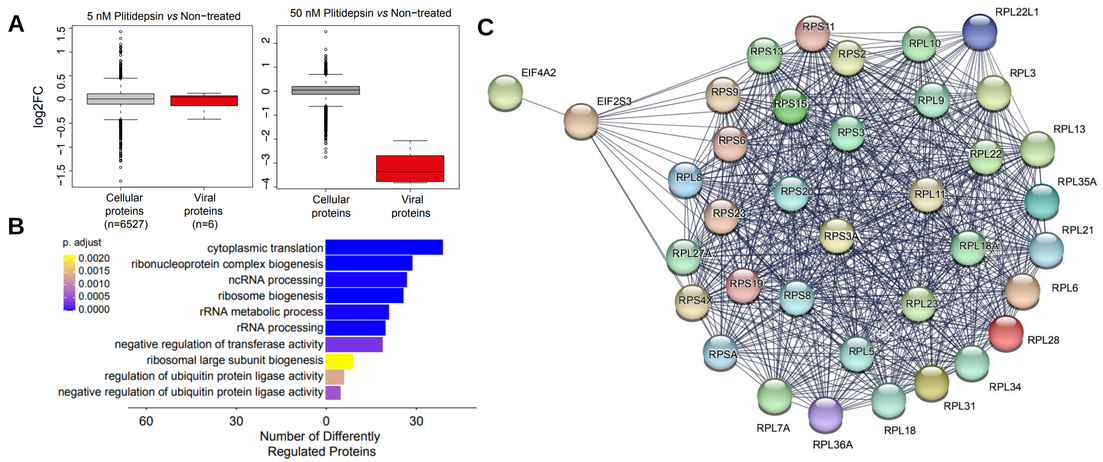

***Fig. 2. A proteostatic mechanism induced by plitidepsin rescues translation of cell proteins**. **A**. Changes in cell and viral proteome in the presence of plitidepsin (treated vs. non-treated cells). FC: fold-change. **B**. Number and biological ontogeny of differentially up-regulated cell proteins in plitidepsin-treated cells. **C**. Functional protein-protein interaction network of differentially up-regulated proteins belonging to the Gene Ontology process 'cytoplasmic translation' in cells treated with 50nM of plitidepsin.*

However, the compensatory proteostasis induced by plitidepsin also explains why this drug failed to inhibit the replication of integrated DNA proviruses such as HIV-1 (**Fig. 3B-C**).

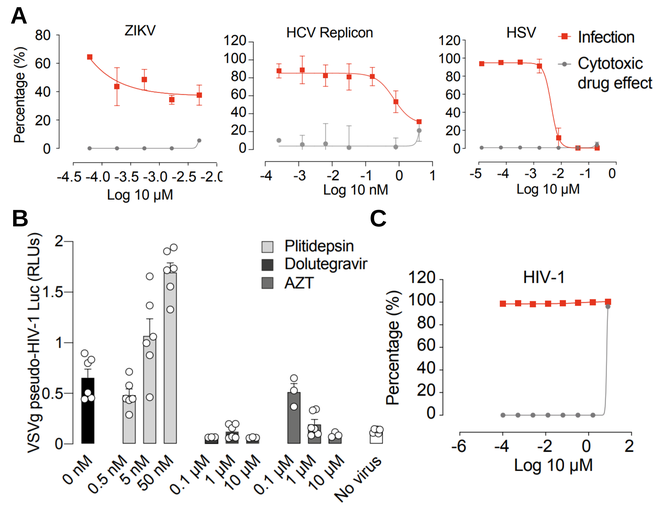

***Fig. 3 Plitidepsin action against different RNA and DNA viruses**. **A**. Dose-response curve of plitidepsin against the indicated viruses (red lines) and cytotoxic effect of the drug in the absence of virus (grey lines). ZIKV: Zika virus; HCV: hepatitis C virus; HSV: herpes simplex virus. **B**. Infection of a luciferase single-cycle HIV-1 construct pseudotyped with VSVg in the presence of the indicated antivirals. RLU: relative light units. AZT: zidovudine. **C**. Dose-response curve of plitidepsin against HIV-1 (red line) and cytotoxic effect of the drug in the absence of the virus (grey line).*

CONCLUSIONS

Unraveling the **mechanism of action** of **host-directed** therapies like **plitidepsin** is imperative to define the indications and antiviral profile of these compounds. This knowledge will be key to develop **broad-spectrum treatments** and have them ready to deploy when future pandemic viruses break through.

Funding

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Broad-Spectrum Antivirals Study Group: Daniel Perez-Zsolt, Elisa Molina Molina, Joan Josep Bech-Serra, Roger Badia, Eva Riveira-Muñoz, Edurne Garcia-Vidal, Martin Sachse, Marçal Gallemí, Jordana Muñoz-Basagoiti, Sandra Franco, Sara Y. Fernández-Sánchez, Dàlia Raïch-Regué, Cristina Lorca-Oró, Raquel Tenorio, Isabel Fernández de Castro, Jorge Carrillo, Julià Blanco, Alejandro Losada, Pablo Avilés, Carmen Cuevas, Júlia Vergara-Alert, Joaquim Segalés, Miguel Ángel Martínez, Roger Paredes, Bonaventura Clotet, Cristina Risco, Ester Ballana, Carolina de la Torre and Nuria Izquierdo-Useros.

Corresponding author: NI-U (nizquierdo@irsicaixa.es)