NEWSLETTER

IUPHAR Mediterranean Group of Natural Products Pharmacology

Plitidepsin has potent preclinical efficacy against SARS-CoV-2 by targeting the host protein eEF1A

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STUDY DESIGN Preclinical *in vitro* and *in vivo* study

TREATMENT Plitidepsin (from *Aplidum albicans*)

Main results and author's conclusion

The Authors of this paper describe the preclinical anti-SARS-CoV-2 efficacy of the drug plitidepsin, a cyclic depsipeptide discovered in the Mediterranean Sea tunicate *Aplidum albicans*, already marketed as Aplidin® in Australia for the treatment of multiple myeloma (MM). The results obtained showed an anti-SARS-CoV-2 activity with an IC_{90} of 1.76 nM in monkey cells, and an even better IC_{90}, of 0.88 nM in human cells (approximately 30 times more potent than remdesivir). Also tested in a pneumocyte-like human cell model, the marine drug showed an antiviral IC_{90} of 3.14 nM. The exogenous overexpression, in host cells, of the eukaryotic translation elongation factor eEF1A, in its mutated form eEF1A-A399V, reduced the antiviral effect of the drug more than 10 times, indicating that the wild-type protein, already known to be targeted by the drug in MM, is relevant also for the antiviral efficacy. On the other hand, the expression of the mutated protein did not significantly influence cell replication, confirming literature evidence on the greater sensitivity of coronavirus than host cells to gene translation perturbation. The role of eEF1A in the antiviral mechanism of plitidepsin was confirmed by the Authors through gene knock-down, experiments too. Moreover, the study included an interesting *in vivo* investigation in two mouse models of SARS-CoV-2 infection. In these models, 0.3 mg/kg of plitidepsin for three consecutive days, or 1 mg/kg for a single day, the first or the single dose being given 2 hours before infection, significantly reduced the viral titer in the lungs. In conclusion, plitidepsin is suggested as a promising therapeutic candidate for COVID-19.

Commentary

The emergency related to COVID-19 makes necessary the search for new molecules active against SARS-CoV-2 infection, capable of being launched into clinical trials in a short time. Molecules active against SARS-CoV-2, already marketed for other diseases and therefore already evaluated in clinical trials for safety and pharmacokinetics, have had a preferential access to further experimentation in patients. Plitidepsin is one of these drugs, developed by Pharmamar (Spain) for the treatment of MM, and currently already entered in a phase III study, started in June 2021 (estimated end in November 2021), for the evaluation of efficacy in adult patients with moderate COVID infection, in association with a dexamethasone treatment (Neptun study, NCT04784559). The target of plitidepsin, the eEF1A factor, which was already proved to be important for the replication of many viruses, is confirmed by the study described as a useful target in counteracting SARS-CoV-2 infection. By virtue of the target, this drug can also be considered effective against SARS-CoV-2 variants. If the clinical trial confirms the efficacy in already infected patients, a new weapon against SARS-CoV-2 will be available and a new chapter will open for the pharmacology of antivirals and the search for new natural compounds targeting eEF1A or other proteins with a similar role in virus replication.

Three chemotypes of thyme (*Thymus vulgaris L.*) essential oil and their main compounds affect differently the IL-6 and TNFα cytokine secretions of BV-2 microglia by modulating the NF-κB and C/EBPβ signaling pathways

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STUDY DESIGN Preclinical *in vitro* study

TREATMENT Three chemotypes of *Thymus vulgaris* essential characterized to contain as main component geraniol (54.9%), thujanol (33.9%) and linalool (69.2%). In comparison, pure compounds were also tested.

Main results and author's conclusion

GC-MS analysis detected linalool, geraniol, and thujanol as the main compounds in three thyme essential oil chemotypes. The results show that the cells maintain their viability after 24h treatment either with chemotypes or standards at 200-folds dilution. BV-2 murine microglial cells were cultured for 24h and then treated with the essential oils or standards. The inflammatory condition was induced by LPS treatment. The anti-inflammatory and neuroprotective activity of the natural compounds was analyzed in three experimental setups: LPS pretreatment for 24 h followed by essential oil treatment for 24 h; essential oil pretreatment for 24 h then LPS treatment for 24 h; and co-treatment with LPS and essential oils for 24 h. Thyme essential oil chemotypes decreased the mRNA expression of IL-6 and TNFα, meanwhile, thujanol showed no significant change. In this study, the effect of essential oils on NF-κB (p50/p65) and P-C/EBPβ pathways using specific antibodies against p50 and p65 was also evaluated. Both geraniol chemotype and standard, as well as thujanol standard were able to decrease NF-κB levels. Similar effects were obtained on P-C/EBPβ transcription factor with geraniol and thujanol standard, as well during the LPS pretreatment. The protective effects seem to be due to different components in comparison to those able to act as antibacterial. Results highlight that chemotypes are more effective than standards but some of the latter can act more effectively on single neuroinflammatory pathways.

Commentary

It has been recognized that neuroinflammation has a significant role in the development of diseases like Alzheimer disease, Parkinson disease, or multiple sclerosis. Among all-natural products the essential oils are good candidates and have a strong literature background. In this study, the Authors suggest *Thymus vulgaris* essential oil chemotypes and main compounds for their neuroprotective and anti-aging effects. They have been proposed as a complementary therapy in neurodegenerative related diseases. From a pharmacokinetic point of view, the essential oils are lipophilic and can move through the nasal mucosa, indeed cross the blood-brain barrier; nevertheless, the components could have different permeability and have distinct targets. This study showed that thyme essential oil works as a multi-target and has suggested that *Thymus vulgaris* could decrease the levels of proinflammatory cytokines. Ultimately, this paper supports the need for further experiments (in vitro and in vivo) to highlight which compounds of the essential oil and the correct strategy (preventive or therapeutic) are more suitable.

Antiviral Activity of *Vitis vinifera* Leaf Extract against SARS-CoV-2 and HSV-1

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STUDY DESIGN Preclinical *in vitro* study

TREATMENT *Vitis vinifera* leaf extract characterized in polyphenols

Main results and author's conclusion

Grape (*Vitis vinifera*) is cultivated worldwide and contains various compounds with acknowledged phytochemical and pharmacological properties, including hepatoprotective, hypoglycemic, cardio-protective, antioxidant, antimicrobial, and antiviral activities. Among the different parts of the plant, pomace, skins, and seeds are of particular interest as wine-making industry by-product. However, there is still lack in scientific literature about the use of leaves as sources of active biomolecules. In this regard, Zannella and Colleagues (2021) have recently described the polyphenolic composition and the antiviral properties of an alcoholic extract from *V. vinifera* leaves. Particularly, the authors focused their attention on the antiviral effects of the extract against Herpes simplex virus type 1 (HSV-1) and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). HSV-1 and SARS-CoV-2 were propagated in Vero cell line (ATCC CCL-81) and the extract (0.625-500 µg/mL) antiviral activity was measured in the following conditions: (i) co-treatment; (ii) virus pre-treatment; (iii) post-treatment; (iv) cell pre-treatment. In parallel, the gene expression of HSV-1 UL27 and SARS-CoV-2 spike protein, both involved in the binding of viral envelope with the cell membrane, was assayed. The qualitative phytochemical analysis led to the identification of about 40 polyphenolic compounds, among which luteolin, kaempferol, apigenin, isorhamnetin, myricetin, chrysoeriol. The extract was able to block the replication of both viruses, in Vero cells. Specifically, the co-treatment and virus pre-treatment paradigms were the most efficacious, with an almost complete inhibition starting from the concentration of 10 µg/mL. The inhibition of HSV-1 and SARS-CoV-2 replication was mirrored by the concomitant inhibition of the gene expression of UL27 and spike protein, respectively.

Commentary

Overall, the study highlights the potential antiviral properties of *V. vinifera* leaf extract, possibly related to the polyphenolic fraction. In this context, the results also highlight how is important to explore the potential of high-quality byproducts, which could represent not only valuable sources of biomolecules with health-promoting interest, but also an innovative approach in sustaining local botanical chains.

A non-pharmacological therapeutic approach in the gut triggers distal metabolic rewiring capable of ameliorating diet-induced dysfunctions encompassed by metabolic syndrome

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STUDY DESIGN Preclinical *in vivo* study

TREATMENT Policaptil Gel Retard®, a medical device constituted by a complex of polysaccharides, EU patent Nr EP1679009 (2006). It is system of molecules of natural origin assembled in order to achieve typical emerging properties such as enhanced water binding (WBC) and swelling capacity (SC) associated to the capability of sequestering lipids and carbohydrates from the diet.

Main results and author's conclusion

In the present study, mice were fed a high fat diet (HFD) in order to develop metabolic alterations similar to those determined by the metabolic syndrome in humans. Animals were treated with a system of molecules of natural origin rich in polysaccharides and mucilages assembled to achieve typical emerging properties such as enhanced water binding (WBC) and swelling capacity (SC). Such properties are associated to the capability of sequestering lipids and carbohydrates from the diet. Treatment reduced the increase of body weight and macroparameters such as oral glucose tolerance test, insulin tolerance test and serum triglycerides. A circadian study of liver gene expression revealed mechanistic aspects underlying the observed efficacy. Following administration of the system of molecules, a profound remodeling of hepatic metabolic activity throughout the day was observed. This was characterized by recovery of insulin sensitivity, glucose homeostasis and a correctly fluctuating circadian rhythm. Generation of *in silico* models based on these data allowed to predict, and subsequently experimentally validate, a reduction in glycogen and triglyceride accumulation in the liver and compensation of free insulin-like growth factor binding protein-2 levels. Taken together, the results suggest the onset of profound metabolic effects on a systemic scale, obtained via the key contribution of a non-pharmacological activity exerted in the intestinal lumen. In order to achieve a more detailed appreciation of the primary phenomena induced by treatment in this anatomical district, fecal lipidic and carbohydrate content as well as microbiota composition were investigated. The analysis revealed a compensation of the Firmicutes/Bacteroidetes ratio (a typical alteration also in the clinical setting) as well as an enhanced excretion of carbohydrates and lipids. It was therefore possible to identify a prebiotic activity and sequestering of diet nutrients as fundamental mechanisms evoked by the system of molecules in order to induce a profound remodeling of metabolism on a systemic scale.

Commentary

This study offers relevant point of reflection in conceptual, technical and regulatory terms. Conceptually, it underlines the importance of searching novel therapeutics activities by maintaining a systemic perspective, based on the idea of a network of multiple, interconnected events. The study allows to appreciate how crucial it is to avoid operating in an exclusively reductionist fashion, trying to reconduct management of a complex syndrome to single events such as the regulation of a single target. Such an approach is certainly applicable to many other pathophysiological contexts. From

an exquisitely technical standpoint, the study supports the idea that the application of a systemic perspective is indeed sustained by the use of -omic techniques and *in silico* modeling tools. It is also possible to argue that such techniques are particularly useful to explore the mechanism of action of complex substances such as those of natural origin, which represent a field of application where the classical key-lock models usually applied to the investigation of single molecules, result limited. Finally, the study highlights the observation that the classification of natural complex substances as medical device, therefore exerting a non-phamacological activity according to EU regulation 2017/745, represents a great opportunity to develop innovative therapeutic solutions based on natural complex substances, demanding the certification of their efficacy and safety profile according to highest standards.