

Violacein, A Microbial Antiviral Product: Does Play Key Role as Active Agent Against SARS-CoV-2?

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Abstract

Violacein (3-(1,2-dihydro-5-(5-hydroxy-1H-indol-3-yl)-2-oxo-3H-pyrrol-3-ilydene)-1,3-dihydro-2H-indol-2-one), a microbial product from *Chromobacterium violaceum* was characterized after continuous attempts to feature it, based on degradation and synthesis procedures, at the University of Liverpool (England), from 1958 to 1960 and only only at 2001 was chemically synthesized. It is a quite known antimicrobial and antiviral natural product. New attempts to solve the infection caused by, or find the proper therapy for, SARS-CoV-2 or producing the infection called COVID-19, must adopt multidisciplinary approach. The aim of the current study is to address the targets, possible strategies and perspectives of new technologies and therapies on SARS-CoV-2. It also hypothesizes the potential of using the therapeutic drug called violacein as multifunctional agent to treat patients at different SARS-CoV-2 contamination stages. Our experience and knowledge about violacein has led us to extrapolate the potential use of this pigment. Violacein's multiple biological activities as also knowledge on its toxicity and antiviral activity enabled suggesting that it could be the new important agent used to treat SARS-CoV-2. Violacein is highly likely to act as protease inhibitor, at ACE-2 receptor level and as immunotherapeutic drug against COVID-19. In term of chemotherapy it will be discussed the actual antiviral used against SARS-CoV-2, such as, thalidomide, ivermectin and melatonin, among others.

Keywords: Genetic Variants, ACE2, SARS-CoV-2, Coronavirus, Human Populations

Introduction

General Aspects

Pigmented microorganisms called small organisms were discovered in 1867, but only in 1881 they were reported by Boisbaudran¹ as pigmented organisms. Bergonzini (1881)² has accidentally found a very dense film of violaceum color that was initially called *Cromococcus violaceum*. After conducting some tests, Bergonzini (1981)² has classified it as bacterium and called it *Cromobacterium violaceum*. In the same year, Zimmerman (1881)³ has changed its name from *Cromo bacterium* (provided by Bergonzini) to *Chromobacterium*. Later on, Gessard (1882)⁴ has observed this pigment type in other bacterial species. The classification as “*Bacillus violaceus*”, which was used from 1905 to

1934, was no longer used at that time. Only in 1967, based on results published by Boisbaudran,¹ De Moss⁵ has suspected that it could be the *Chromobacterium violaceum* and the pigment, violacein. Nowadays, genus *Chromobacterium* was classified by Sneath⁶ (references therein)⁷. A violet bacterium was found by UFRJ Prof. W.C. de Araujo in water treatment station in Manaus City (Amazonas State-Brazil) in 1976; it was identified as *Chromobacterium violaceum*. The ecological behavior of the violaceous pigment (violacein) (3-(1,2-dihydro-5-(5-hydroxy-1H-indol-3-yl)-2-oxo-3H-pyrrol-3-ilydene)-1,3-dihydro-2H-indol-2-one) was subsequently explained by Caldas.^{8,9}

Violacein structure was finally defined after continuous

attempts to feature it, based on degradation and synthesis procedures, at the University of Liverpool (England), from 1958 to 1960.¹⁰ Wille and Steglich¹¹ have reported violacein's chemical synthesis 20 years ago (Figure 1)

Since then, violacein has been the object of great academic interest due to its remarkable biological and physical properties.¹²⁻²⁴ Besides its well-known action

on different bacterial species, violacein can be used to treat several diseases caused by fungi, parasites, and even cancer, as reported in the literature. Several studies available in the literature have focused on investigating the biomedical application of bacterial pigments, such as violacein, whereas new opportunities and challenges to the use of these biopigments have been emerging in the scientific field.²⁵⁻²⁷

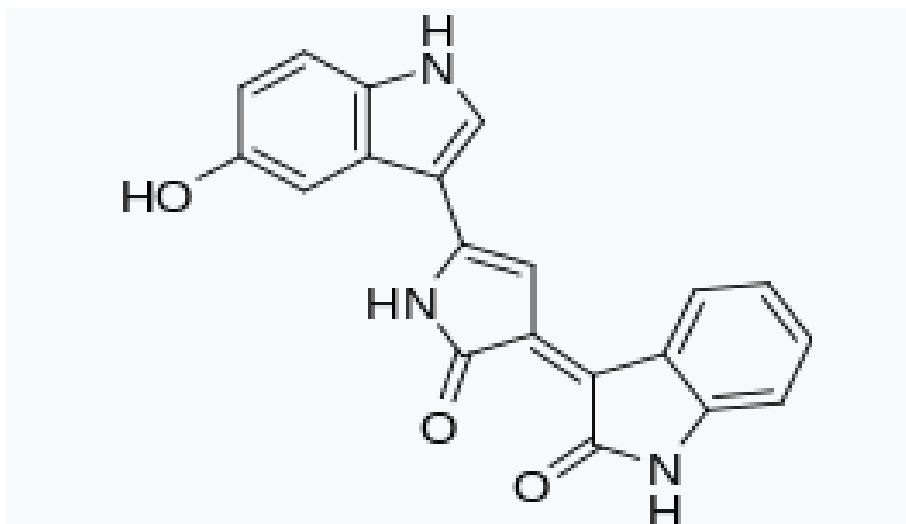


Figure 1. Violacein Structure.

Toxicity

Concerns about the pharmacological potential of violacein have led the scientific community to assess its toxicity in studies carried out *in vitro* and *in vivo*. The first studies about violacein cytotoxicity were conducted with V79 fibroblasts based on different endpoints.^{28,29} Based on assays such as tetrazolium salt reduction, nucleic acid level and neutral red uptake, as well as on violacein cytotoxicity, IC_{50} (cell viability inhibition at 50%) ranged from 5 μ M to 12 μ M and resulted from apoptosis.^{28,29} Based on tetrazolium blue reduction test, violacein has shown decreased human lymphocyte proliferation, after it was stimulated with phytohemagglutinin, at $IC_{50} > 10 \mu$ M.³⁰ At this point, it is important emphasizing that increased violacein cytotoxicity was observed in tumor cells in comparison to that observed in lymphocytes, mainly when different hematologic cancers were taken into consideration.^{17-19,25} However, violacein cytotoxicity in other non-tumorigenic cell lines has been investigated, as well. For example, violacein cytotoxicity and its action mechanism were investigated in MRC-5 (human fetal

lung fibroblast) cells by Leal et al.,³¹ whose results have shown that cell viability was higher than 60% after 24-h exposure to 6 μ M violacein. On the other hand, MRC-5 cell viability has decreased to 50% after treatment with 3 μ M violacein, for 48 h. In addition, studies have suggested that both apoptosis and necrosis are involved in the cell death mechanism. The increased mitochondrial membrane potential has indicated that violacein-induced cell death is associated with mitochondrial damage caused by membrane hyperpolarization.³¹ Interestingly, a series of studies conducted with living yeast cells has shown that 20 and 40 μ M violacein concentrations did not disrupt membrane integrity in yeast species *Saccharomyces cerevisiae*, since changes in membrane potential ($\Delta\Psi$) were not observed in it.³² Fluorescence microscopy technique has evidenced that violacein was capable of permeabilizing *B. subtilis* and *S. aureus* cells (Gram-positive bacteria). The permeabilization process was followed by the emergence of visible gaps or ruptures in cytoplasmic membrane, although they did not affect cell wall. These data have shown that the cytoplasmic membrane

was violacein's initial target in the analyzed bacterial specimens.³³

However, violacein has also led to dose-dependent oxidation of pyridine nucleotides in yeasts, as well as to small oxygen uptake increase and respiratory chain disturbance, based on oxidation-reduction process observed in cytochrome c.³² It is worth emphasizing that violacein genotoxicity was assessed in several mammalian cell lines (HEp-2, VERO, FRhK-4, and MA104) based on Comet assay; VERO cell line was assessed through micronucleus assay. Although significant DNA damage was not observed in MA104 and HEp-2 cells, violacein has led to significant DNA cleavage in FRhK-4 cells and induced micronuclei in VERO cells, at dose-dependent response level. These outcomes indicate that violacein has shown genotoxicity in FRhK-4 and VERO cells.³⁴

It is essential highlighting a 35-day study about violacein toxicity in mice, which was based on total hematology, biochemistry analyses such as AST, ALT, and creatinine content, as well as on the histopathological analysis of organs such as kidney and liver. The aforementioned study has evidenced that violacein administration up to 1,000 µg/kg on a daily basis for 35 days was well tolerated by mice and that it did not induce hemato-, nephro- or hepatotoxicity when it was intraperitoneally applied to them. Moreover, this study was one of the first publications about violacein antitumor activity *in vivo* that did not show any toxicity in models' main organs.³⁵ It opened room for further studies on violacein's pharmacological properties *in vivo*, such as antiplasmodial, anticancer, anti-inflammatory and immunomodulatory activity.¹⁹ Pauer et al.,³⁶ have recently investigated violacein effect on gut microbiota. Their study was based on oral violacein administration to male Wistar rats, at concentrations of 50 µg/ml (low) and 500 µg/ml (high), for 30 days. Administration of low violacein doses has shifted the composition of bacterial communities, where bacterial classes *Clostridia* (Firmicutes) and *Bacilli* prevailed, and brought benefits to hosts affected by syndromes associated with inflammatory diseases. High *Bacilli* concentration was followed by Actinobacteria and *Clostridia*, which prevailed as major elements in gut microbiota. The aforementioned study has provided the framework for new therapies focused on intestinal diseases.³⁶

Study focused on investigating trypanosome infection in male albino mice (18-20 g) intraperitoneally injected

with up to 100 mg/kg of violacein for a week did not observe death or visual toxicity effect on them.²⁸ Study *in vivo* focused on investigating violacein immunomodulatory, analgesic and antipyretic effect on models intraperitoneally injected with up to 40 mg/kg of violacein did not observe toxic effect on them.³⁷

Rats treated with 20 and 40 mg/kg p.o of violacein have shown enhanced kidney function, as well as reduced high uric acid, creatinine and blood urea levels, in comparison to the control.³⁸

Female C57BL/6 mice were i.p. injected with violacein doses ranging from 1.75 mg/Kg to 7.00 mg/Kg, on a daily basis, for 3 successive days, in order to check drug toxicity. Mice treated with 7 mg/kg of violacein have died after the second dose application, whereas doses up to 3.5 mg/kg were not toxic to this model.³⁹

Antiviral Activity

Violacein antiviral activity has been neglected for several years. The current section will report the great violacein potential to be used against dangerous viruses affecting populations at global scale.

Violacein (10% deoxyviolacein) has effectively acted against Polioviruses and Herpes Simplex Virus (HSV) post-infection of HeLa cells.^{7,21,40,41} Its application at concentration of 0.25 µg/ml has suppressed HSV by 62%, whereas its application at concentration of 0.063 µg/ml has suppressed poliovirus-infected HeLa cells by 56%. These outcomes have suggested significant violacein antiviral activity.

Andrighetti-Frohner et al.,⁴² have also analyzed the violacein cytotoxic and antiviral effects on several virus strains. Violacein application at concentrations not capable of inhibiting cell growth did not show cytopathic or antiviral activity against HSV-1 (29-R/acyclovir-resistant strain); against two different hepatitis viruses such as HAF-203 and HN175 strains; and against adenovirus type 5. However, the MTT assay has shown one-weak suppression of HSV-1 (KOS-1.25 µM [22%]), ATCC/VR-733 strains (0.625 µM [9%]), poliovirus type 2 (1.25 µM [9%]) and simian rotavirus SA11 (1.25 µM [24%]) replication in cell cultures. These data differ from those reported by May et al.,^{40,41} likely because they used different protocols and strains, or due to violacein purity level.¹⁷

Several patent applications have reported violacein activity against different viruses. In addition to the aforementioned study carried out by May et al.,^{40,41}

new studies have reported cyclodextrin/violacein nanostructure,⁴³ as well as pharmacological use of cyclodextrin-Au-thiol-derivative/hydrophobic nanoparticles⁴⁴ as antiviral drug. Cosmetic formulation based on the association between violacein and lipophilic materials was developed to help protecting individuals' skin and (semi) mucous membrane against viruses.^{45,46} *Janthinobacterium lividum* (violacein producer) application has minimized the number of microbes in, and/or maximized the therapeutic effects as antimicrobial agent on, a given host and/or host area. The process comprised at least one virus, besides other organisms.⁴⁷

Eliminating virus vectors is an effective strategy applied to eliminate viruses. Indirect action by genus *Chromobacterium* on dengue, chikungunya and Zika virus vectors during *Aedes aegypti* treatment was reported in the literature. *Chromobacterium subtsugae* (violacein producer) has shown insecticidal action against distinct insect types, such as beetles, whiteflies and moths.⁴⁸ *Chromobacterium vaccinii* (violacein producer) presented insecticidal properties, whereas *C. subtsugae* is often used as biocontrol agent called Grandevo® (Marrone BioInnovations).⁴⁹⁻⁵² *C. vaccinii*, added to larval breeding water, was capable of killing *Aedes aegypti* (Zika and dengue virus vector) larvae.⁵¹ *C. subtsugae* has led to high *Aedes aegypti* mortality rate.^{53,54}

What is the Strategy Adopted for Potential Drugs Against SARS-COV-2?

Studies have reported SARS-CoV-2 life cycle in infected cells and its most important infection stages. RBD (receptor-binding domain) of SARS-CoV-2 spike proteins S1 subunit and S2 subunit plays the role of fusing viral and host membranes.⁵⁵ The S1 subunit of SARS-CoV-2 S protein identifies, and binds to, cell receptor ACE2 (angiotensin-converting enzyme 2), as well as to HR1 (heptide repeat 1) and HR2 (heptide repeat 2), in order to form a six-helix bundle to enable virus-cell membrane fusion. SARS-CoV-2 irrupts into target cells through endocytosis or membrane fusion. The viral RNA in the cytoplasm becomes exposed after the virus enters the human cell. Next, polyproteins phosphatase 1 α (pp1a) and protein phosphatase 1 β (pp1ab) are translated and divided to form a replication transcription complex. Full-length (-) RNA copy of the genome produced by the complex is generated during the replication process, based on a full-length (+) RNA genome template. Sub-genomic RNA capable of encoding structural proteins is generated during transcription. The nucleocapsid of the virus is formed by genomic RNA and N proteins found in the cytoplasm; then, it sprouts into the ERGIC (endoplasmic reticulum (ER)-Golgi intermediate cavity) lumen. Next, viral particles are released from contaminated cells due to exocytosis⁵⁶ (Figure 2).

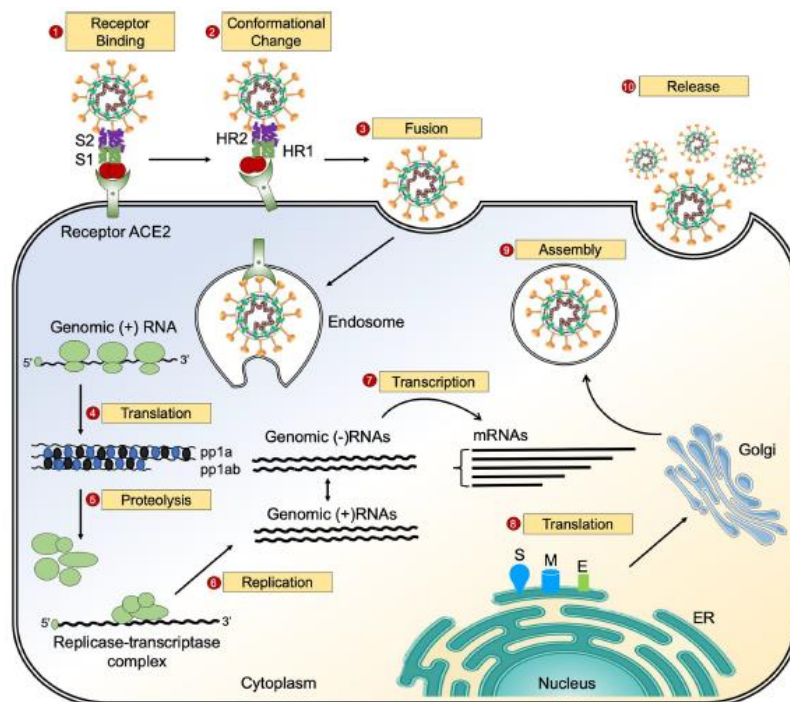


Figure 2. SARS-CoV-2 Life Cycle in Host Cells (extracted from Li et al.⁵⁶ with permission by John Wiley & Sons Ltd.)

Possible Targets

RBD of SARS-CoV-2 Spike Protein

As previously mentioned, the RBD of SARS-CoV-2 spike protein plays decisive role in binding the ACE2 receptor necessary to enable viral infections. Several strategies to target the RBD-ACE2 binding profile and to delineate RBD targeting vaccines and medicines have been investigated. Results have shown that these structures are approximately 10 nm far from RBD and SARS-CoV-2 polybasic disrupted sites. There was increase in RBD-ACE2 binding affinity via hydration and electrostatic interactions. Negatively charged tetrapeptide (GluGluLeuGlu) was indicated to neutralize the positively charged spike protein (arginine) in polybasic disruption sites. This peptide bound to one polybasic disruption site of the SARS-CoV-2 spike protein and evidenced the viability of neutralizing the RBD-ACE2 binding. This outcome helps better understanding the SARS-CoV-2/ACE2 binding mechanism in order to substantiate new therapeutics for COVID-19 infection.⁵⁷

M^{pro} Protease

SARS-CoV-2 protease inhibitors are viable options if one takes into consideration several studies, since the main protease (M^{pro}, also called 3CL^{pro}) suppressor is a potential target for this action. SARS-CoV-2: M^{pro} is a crucial coronavirus enzyme that plays essential role in modulating viral transcription and replication processes, a fact that turns it into an appealing target drug for SARS-CoV-2. Several drugs and inhibitors were featured based on computer aided, structure-based and cell-based medicine project strategies.⁵⁷ Protease inhibitor “Ebselen” has shown encouraging antiviral activity in cell-based tests, among them.⁵⁸

M^{pro} suppressor Lopinavir/Ritonavir was analyzed but it did not show any benefit to severely-ill COVID-19 patients subjected to standard care.⁵⁷ The FDA-approved chloroquine and hydroxychloroquine (HCQ), in association with azithromycin, did not show any clinical improvement at the 15th day of treatment in comparison to the standard care.^{20,57}

Remdesivir has acted in the RNA-dependent RNA polymerase and inhibited viral RNA synthesis. However, severe adverse incidents were observed and remdesivir was ineffective even among severely-ill patients.⁵⁶

Immunopathology

In immunopathology terms, SARS-CoV-2 reduces or

suppresses T-cell count, increases pro-inflammatory CD⁺ Th17, CCR4⁺, CCR6⁺ cell secretion, as well as IFN- γ , IL-1, -4, -6, -10, IP-10, and MCP1 secretion, produces cytokine storm and increases TNF- α , MIP-1A, IL-2, -7, -10, G-SCF, IP-10, and MCP-1 serum secretion.⁵⁶

Besides its antiviral action,⁵⁹ nitazoxanide can suppress the generation of pro-inflammatory cytokines such as TNF, IL-2, -4, -5, -6, -8, and -10 in PBMCs (peripheral blood mononuclear cells). Mice orally exposed to nitazoxanide application *in vivo* have shown plasma IL-6 contents significantly reduced by over 90% related to vehicle as control used in mice. Although the relevance of all nitazoxanide-related outcomes to humans is yet to be investigated, data available in the literature indicate that this drug likely enhances outcomes in patients contaminated with MERS-CoV by inhibiting the excessive production of pro-inflammatory cytokines such as IL-6.⁶⁰

A Brazilian multicenter, randomized, double-blind, placebo-controlled trial was conducted with adult patients presenting up to three-day post-onset of COVID-19 symptoms (Brazilian Registry of Clinical Trials (REBEC) number RBR-4nr86m and Clinical Trials.gov number NCT04552483). After RT-PCR confirmation, patients were randomized (1:1) to receive either 500 mg of nitazoxanide or placebo, three times a day (TID), for 5 days. Primary results focused on full symptom inhibition. Secondary results comprised viral content or load, serum inflammation biomarkers, laboratory assays and, finally, hospitalization rate. Negative cases were also evaluated. Patients treated with nitazoxanide presented viral load decrease by 55% from the beginning to the end of therapy, whereas patients treated with placebo presented viral load decrease by 45%. However, after five treatment days, there was no difference in symptoms between mild COVID-19 patients treated with nitazoxanide and placebo.⁶¹

Chemotherapy

Thalidomide

Studies carried out *in vitro* or *in vivo* have shown that thalidomide impaired TNF- α (tumor necrosis factor alpha) production, as well as increased IL-12 levels, the number of peripheral blood CD8⁺ T cells, IFN- γ production and cytotoxic effect on cultures cells. Based on the study carried out *in vitro* by Tabata et al.,⁶²

thalidomide was capable of diminishing IL-6 and IL-1 β expression in human lung epithelial cells and helped avoiding emphysema.²⁰

Besides suppressing cytokine release and modulating immune roles, thalidomide was also used to relax COVID-19 patients to help decreasing their oxygen uptake and relieving digestive symptoms. Thus, thalidomide may shine new light on adjuvant therapeutic strategies focused on fighting this lethal viral infection. At this point, it is necessary conducting randomized controlled trials focused on investigating the effectiveness of thalidomide application, in association with low glucocorticoid concentrations to treat COVID-19 pneumonia.⁶³

Ivermectin

Caly et al.,⁶⁴ have shown that ivermectin presented antiviral action *in vitro* against clinical SARS-CoV-2 isolates; a single ivermectin application was capable of controlling viral load increase within 24-48 h. It was suggested that it may have happened due to suppression of IMP α / β 1-mediated nuclear import of viral proteins, as evidenced in several RNA viruses. The SARS-CoV-2 action mechanism, and the identification of specific

SARS-CoV-2 and/or host-influenced components, are pertinent topics to be investigated in future research in this field (Figure 3).

Clinical trials have recently highlighted the important role played by ivermectin in COVID-19 treatment; however, it is necessary collecting further evidence based on Randomized Controlled Trials (RCTs) and on dose-response assessments to justify ivermectin application in COVID-19 cases. *In silico*-based analysis performed through artificial intelligence and classical mechanics simulation has indicated ivermectin action in viral protein sites. Thus, studies have suggested that ivermectin was highly effective as antiviral drug; however, its administration was limited due to pharmacokinetic issues such as its poor solubility. These impediments can likely be overcome through the formulation of nanostructured ivermectin or even of other drugs with improved physicochemical properties. There are suggestions that the ivermectin-inhalation therapy can reach high concentrations of it in the lungs and airways in order to diminish viral accumulation in these areas or that it can be used in association with other active ingredients accounting for different action mechanisms.⁶⁵

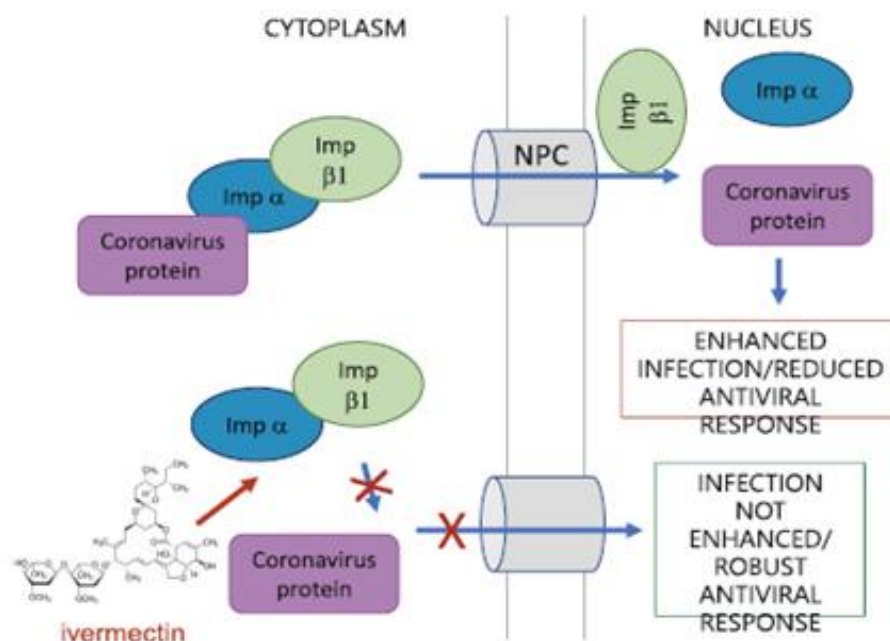


Figure 3. Scheme of Ivermectin's Proposed Antiviral Action on SARS-CoV-2 Virus. IMP α / β 1 binds to the coronavirus carrier protein in the cytoplasm (top) and translocate it through the nuclear pore complex (NPC) into the nucleus; the complex collapses, the viral carrier diminishes the antiviral action of host cells and increases contamination level. Ivermectin binds to, and disrupt, the Imp α / β 1 heterodimer, thus preventing Imp α / β 1 from binding to the viral protein (bottom) and from getting in the virus nucleus. This process likely diminishes antiviral activity suppression and enables normal and highly effective antiviral response (Extracted from Caly et al.⁶⁴ with permission from Elsevier B.V.)

Melatonin

Melatonin (hormone) is another promising agent to be used against SARS-CoV-2. Mitochondria are a major site of peripherally-produced melatonin action, since it is the place where this hormone neutralizes the reactive oxygen species (ROS) generated during oxidative phosphorylation processes. Melatonin also plays major role as immune modulator, since it reduces overreactions to foreign agents and simultaneously boosts immune processes. In addition, it can be used to suppress damages caused by cytokine storm during pandemics such as the coronavirus infection caused by the SARS-CoV-2 virus. Melatonin implications in COVID-19 susceptibility and treatment have been addressed.^{21,66}

The expression of genes relevant to virus invasion and infection can change based on a genic index (MEL-Index) used to estimate lungs' ability to synthesize melatonin. The entry of virus in epithelial AT2 cells should be interfered by an affirmative correlation transmembrane protease serine 2 (TMRPSS2) and with a contrary correlation with the coding gene for cellular endoprotease (furin), indicating dysfunctional alteration in virus spike. Moreover, MEL-Index also has negative correlation to genes accounting for codifying multi-molecular receptor complex CD147 proteins, macrophages' gateway and other immune cells. Thus, the idea that lung and respiratory tract melatonin could be a natural protective factor opens new epidemiological and pharmacological perspectives, since high MEL-Index scores could be predictive of asymptomatic carriers, whereas nasally-administrated melatonin could help preventing the evolution of pre-symptomatic carriers.⁶⁷

Justification of the Hypothesis: Violacein may Play Important Role in COVID-19 Treatment?

Receptor-Binding Domain (RBD)

Since violacein is an antiviral compound with poor solubility in water, polymeric poly-(D, L-lactide-co-glycolide) nanoparticles capable of loading this compound can enhance its solubility and biological behavior. Violacein nanoparticles encapsulated in the polymer presented diameter of 128 ± 14.6 nm and zeta potential of -15.9 ± 0.7 mV (surface charge). Drug release kinetics tests conducted *in vitro* have shown that violacein encapsulated in these nanoparticles presented controlled release behavior up to 5 days, as well as excellent antimicrobial activity.⁶⁸ This negatively-

charged nanostructure could interact with SARS-CoV-2 in a similar way as that suggested by Qiao and de la Cruz⁵⁷ for negative peptides.

Protease inhibitor

One approach could be a protease inhibition as target to M^{pro}, since it was demonstrated that an inhibitor of protease was found by studying the cytotoxic effects of violacein. Death induced in CD34⁺/c-Kit⁺/P-glycoprotein⁺/MRP1⁺ TF1 leukemia progenitor cells was mediated by calpain (calcium-dependent protease-cysteine protease) inhibition and by death-associated protein kinase 1 (DAPK1). Violacein also induced protein kinase A (PKA), protein kinase B (AKT), and pyruvate dehydrogenase kinase (PDK) activation, which was followed by structural changes caused by endoplasmic reticulum stress and Golgi apparatus collapse, as well as led to cell death.⁶⁹ It is possible inferring some similarity between violacein action and that of protease inhibitors such as Ebselen.⁵⁸

Immunopathology

Matrix metalloproteinases (MMP-2 and -9) play important immunopathological role in tumor metastasis due to pro-inflammatory cytokine cleavage. Zymography analysis has shown that violacein has significantly inhibited cytokine (TNF α and TGF β)-mediated MMP-2 activation in MCF-7 breast cancer cell line. MMP-2 plays critical role in inflammatory chemokine secretion.⁷⁰

Violacein application in chemically-induced ulcers has reduced pro-inflammatory cytokine (TNF- α , IL1 β , and IL-6) levels (1.84-fold, 1.95-fold, and 1.45-fold, respectively), as well as increased anti-inflammatory cytokines (IL4 and IL-10) levels (2.69-fold and 2.28-fold, respectively), and growth factor (VEGF, EGF, and HGF) levels (2.90- fold, 2.43-fold, and 2.41-fold, respectively) in comparison to the untreated indomethacin-induced ulcer group. These data have evidenced that violacein treatment was capable of reducing pro-inflammatory cytokines (TNF- α , IL-1 β , and IL-6) and of simultaneously increasing tissue IL-4 and IL-10 levels, which may have contributed to its immunological effect.^{22,37} Violacein is highly likely to act in similar ways as some of the compounds used in SARS-CoV-2 treatment.

Chemotherapy: Melatonin-Like Activity

Increased estrogen levels in human body increases

the likelihood of breast cancer development, whereas regular concentrations of it play significant role in normal cell functioning. Melatonin is popularly used as anti-estrogenic compound, whereas violacein, which is an active secondary metabolite secreted by bacteria, presents strong structural similarity to melatonin. Consequently, its latency can be tested for anti-cancerous activity. Docking and virtual screening were conducted to prove that violacein and similar compounds are more efficient than melatonin in binding to estrogen receptors and that it has the potential to emerge as the leading anti-estrogenic compound to be used in breast cancer treatment. In fact, violacein presented better binding energy than melatonin as anti-estrogenic drug.⁷¹

Implications of the Hypothesis

Our experience and knowledge about violacein has led us to extrapolate the potential use of this interesting pigment. Violacein's multiple biological activities enabled suggesting that it could be the new important agent used to treat SARS-CoV-2. Violacein is highly likely to act as protease inhibitor, at ACE-2 receptor level and as immunotherapeutic drug against COVID-19. Unfortunately, violacein's antiviral action mechanism remains unknown, so far.

Authors' Contributions

ND, GZJ, WJF, GN- Conceptualization, Methodology, Writing-original draft, Review and Editing.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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