AIJR Preprints
Section: Coronavirus
Article Id: 315, Version: 1, 2021
DOI: https://doi.org/10.21467/preprints.315
URL: https://preprints.aijr.org/index.php/ap/preprint/view/315
{Click on above link or DOI to see the latest available version of this article}

NOT PEER-REVIEWED

Version 1: Received: 21 May 2021 / Approved: 27 May 2021 / Online: 27 May 2021

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

Nelson Durán[1,2,*], Giselle Z. Justo[3], Gerson Nakazato[4,*] Wagner J. Fávaro[1]

[1]Laboratory of Urogenital Carcinogenesis and Immunotherapy, Department of Structural and Functional Biology, Universidade Estadual de Campinas (UNICAMP), Campinas, SP, Brazil

[2]Nanomedicine Research Unit (Nanomed), Center for Natural and Human Sciences (CCNH), Universidade Federal do ABC (UFABC), Santo André, SP, Brazil

[3]Departamento de Ciências Farmacêuticas e Departamento de Bioquímica, Universidade Federal de São Paulo (UNIFESP), São Paulo, SP, Brazil

[4]Laboratory of Basic and Applied Bacteriology, Department of Microbiology, Biology Sciences Center, Universidade Estadual de Londrina (UEL), Londrina, PR, Brazil

*Corresponding authors:

ABSTRACT

Violacein, a microbial product 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 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, COVID19, 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 COVID19. It also hypothesizes the potential of using the therapeutic drug called violacein as multifunctional agent to treat patients at different COVID19 contamination stages. Our experience and knowledge about violacein has led us to extrapolate the potential use of this pigment. Violacein 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 COVID19. Violacein is highly likely to act as protease inhibitor, at ACE2 receptor level and as immunotherapeutic drug against Covid19. In term of chemotherapy, it will be discussed the actual antiviral used against COVID19, such as, thalidomide, ivermectin and melatonin, among others.

Keywords: Violacein, antiviral, SARS-CoV-2, immunology

How to Cite:

Duran *et al.*, "Violacein, A Microbial Antiviral Product:Does Play Key Role as Active Agent Against SARS-CoV-2?". *AIJR Preprints*, 315, Version 1, 2021.

1. INTRODUCTION

a) General aspects

Pigmented microorganisms called small organisms were discovered in 1867, but only in 1881 they were reported by Boisbaudran [1] 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 *Cromobacterium* (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 Boisbaundran [1], De Moss [5] has suspected that it could be the *Chromobacterium violaceum* and the pigment, violacein. Nowadays, genus *Chromobacterium* was classified by Sneath [6] ([7] references therein). 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 Steglich [11] have reported violacein's chemical synthesis 20 years ago (Fig.1)

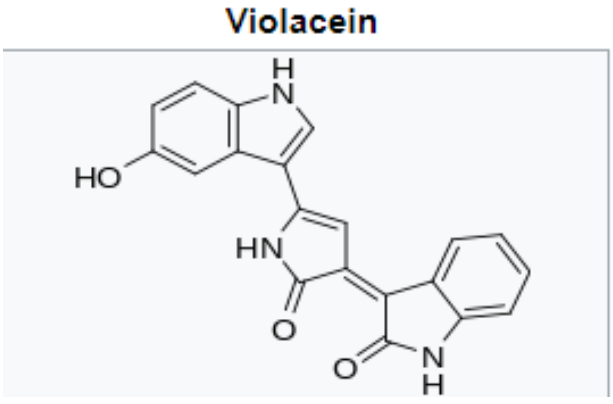

Figure 1: Violacein structure

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].

b) 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, IC50 (cell viability inhibition at 50%) ranged from 5 μM to 12 μM 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 IC50 >10 μM [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, 18, 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 μM violacein. On the other hand, MRC-5 cell viability has decreased to 50% after treatment with 3 μM 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 μM violacein concentrations did not disrupt membrane integrity in yeast species *Saccharomyces cerevisae,* 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].

c) 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 A 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 nanoparticles [44] 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].

2. 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 (hepeptide repeat 1) and HR2 (hepeptide 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 exocytosis [56] (Fig.2)

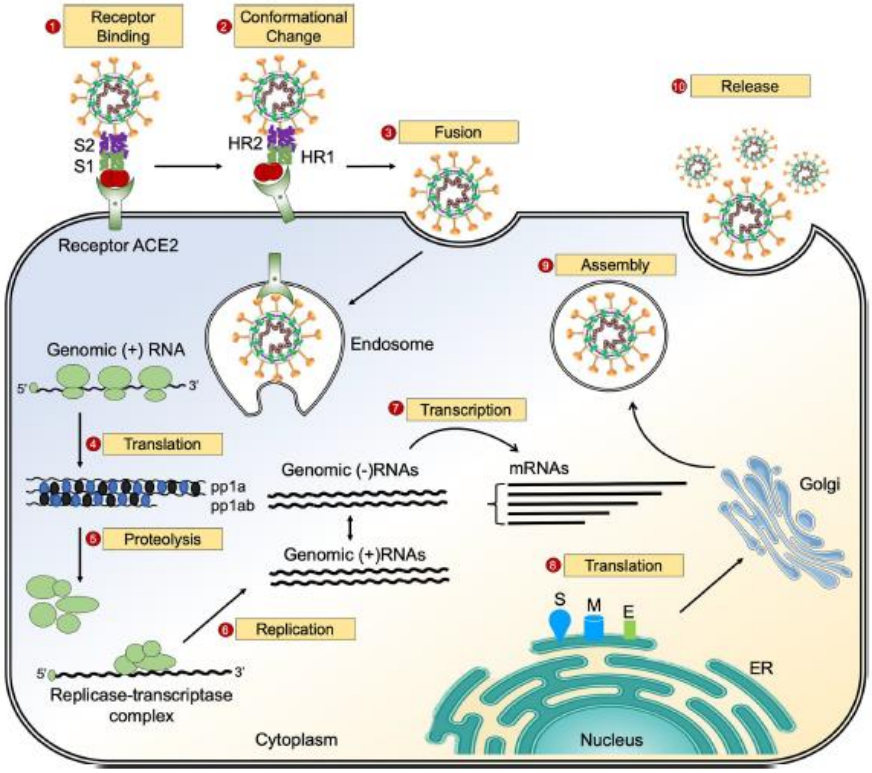

Figure 2: SARS-CoV-2 life cycle in host cells (extracted from Li et al. [56] with permission by John Wiley & Sons Ltd.)

3. POSSIBLE TARGETS

1) 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].

2) **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 15^{th} 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].

3) **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 IFNγ, 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 ClinicalTrials.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].

4) Chemotherapy

4.1) **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-1β 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].

4.2) **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 IMPα/β1-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 (Fig. 3).

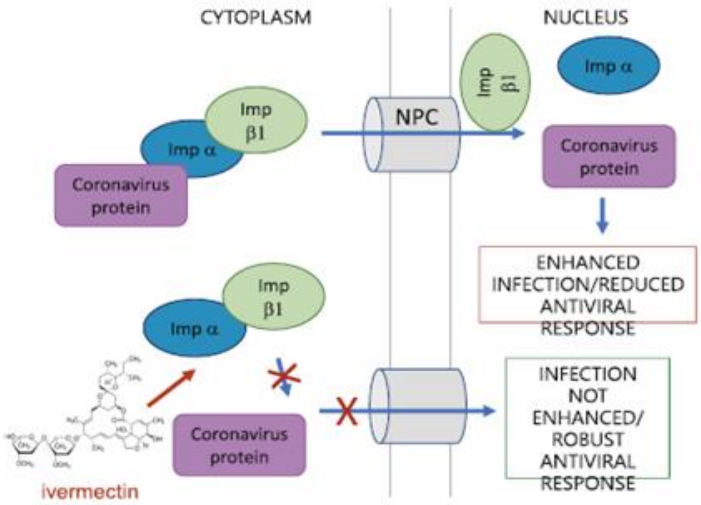

Figure 3: Scheme of ivermectin's proposed antiviral action on SARS-CoV-2 virus. IMPα/β1 binds to the coronavirus carrier protein in the cytoplasm (top) and translocate it through the nuclear pore complex (NPC) into the nucleus; the complex collapses, the viral carrier diminishes the antiviral action of host cells and increases contamination level. Ivermectin binds to, and disrupt, the Impα/β1 heterodimer, thus preventing Impα/β1 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.)

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].

4.3) **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].

5. JUSTIFICATION OF THE HYPOTHESIS: VIOLACEIN MAY PLAY IMPORTANT ROLE IN COVID-19 TREATMENT?

5.1. 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 Cruz [57] for negative peptides.

5.2. 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].

5.3. 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 (TNFα and TGFβ)-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, IL1β, 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-1β, 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.

5.4. 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].

6. 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. However, our research group has already accepted the challenge of implementing a mechanistic study focused on investigating violacein's antiviral action on Herpes Simplex virus (HSV) and on murine Coronavirus.

Author contributions

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

Declaration of Competing Interests

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.

Acknowledgements: The authors would like to thank the São Paulo Research Council (FAPESP grant 2018/10052-1), the Brazilian National Council for Scientific and Technological Development (CNPq grant 552120/2011-1) and Coordinating Agency for Advanced Training of Graduate Personnel (CAPES – funding code: 001).

REFERENCES

[1] Boisbaudran, L. Matière colorante se format dans la calle de farine. Compt Rend Acad Sci 1882; 94: 562-567.
[2] Bergonzini C. Um nuevo pigmento bacterio colorato. Ann Soc Natural, Modena Ser 1881; 2:149-158.
[3] Zimmerman B. Review of Bergozini on Chromobacterium. Bot Cenrtralbl 1881; 4: 1528-1530.
[4] Gessard C. On the blue and green coloration that appears on bandages. Compt Rend Hebdomadaires Seances de L´Acad Sci 1882; 94: 536-538.
[5] De Moss R.D. Violacein. Antibiotics 1967; 2: 77-81.
[6] Sneath PHA. Genus *Chromobacterium* Bergonzini 1881, 153AL, In NH Krieg, JG Holt eds., *Bergey's Manual of Systematic Bacteriology*, Vol. 1, Williams & Wilkins, Baltimore, 1984; p. 580-582.
[7] Durán N, Menck CFM. *Chromobacterium violaceum*: a review of pharmacological and industrial perspectives. Crit Rev Microbiol. 2001; 27: 201-222.
[8] Caldas LR. Photochemistry and photobiology in a virgin land. Photochem Photobiol.1077; 26: 1–2.
[9] Caldas L.R. Um pigmento nas águas negras, Ciência Hoje 1990; 11, 56–57.
[10] Ballantine JA, Beer RJS, Crutchley DJ, Dodd GM, Palmer DR. The chemistry of bacteria. Part VIII. The synthesis of violacein and related compounds. J Chem Soc 1960; 2292–2299.
[11] Wille G, Steglich W. A short synthesis of the bacterial pigments violacein and deoxyviolacein. Synthesis 2001; 5: 759-762.
[12] Azman A-S, Mawang C-I, Abubakar S. Bacterial pigments: The bioactivities and as an alternative for therapeutic applications. Nat Prod Commun 2018; 13: 1747-1754.
[13] Choi SY, Yoon K-H, Lee JI, Mitchell RJ. Violacein: Properties and production of a versatile bacterial pigment. BioMed Res Inter 2015; 105: Article ID 465056.

[14] Choi SY, Im H, Mitchell RJ. Violacein and bacterial predation: promising alternatives for priority multidrug resistant human pathogens. Future Microbiol 2017; 12: 835-838.
[15] Choi SY, Lim S, Cho G, Kwon J, Mun W, Im H, et al. *Chromobacterium violaceum* delivers violacein, a hydrophobic antibiotic, to other microbes in membrane vesicles. Environ Microbiol 2020; 22: 705-713.
[16] Dessaux Y, Elmerich C, Faure D. Violacein: a molecule of biological interest originating from the soil-borne bacterium *Chromobacterium violaceum*. Rev Méd Interne 2004; 25: 659-662.
[17] Durán N, Justo, G.Z., Ferreira, C.V., Melo, P.S., Cordi, L., Martins, D. Violacein: properties and biological activities. Biotechnol Appl Biochem. 2007; 48: 127–133.
[18] Durán M, Ponezi AN, Faljoni-Alario A, Teixeira MFS, Justo GZ, Durán N. Potential applications of violaceína: a microbial pigment. Med Chem Res 2012; 21: 1524-1532.
[19] Durán N, Justo GZ, Durán M, Brocchi M, Cordi L, Tasic L, et al. Advances in *Chromobacterium violaceum* and properties of violacein–its main secondary metabolite: A review. Biotechnol Advan 2016; 34: 1030-1045.
[20] Durán N, Fávaro WJ. The most prominents antiviral pharmaceutics against Covid-19 and their perspectives. Scielo Preprint Posted:2020-06-30.
[21] Durán N, Fávaro WJ, Brocchi M, Justo GZ, Castro GR, Durán M, et al. Patents on violacein: A compound with great diversity of biological activities and industrial potential. Recent Patents Biotechnol 2021; DOI : 10.2174/2213476X07666201221111655.
[22] Justo GZ, Durán N. Action and function of *Chromobacterium violaceum* in health and disease: Violacein as a promising metabolite to counteract gastroenterological diseases. Best Pract Res Clin Gastroenterol 2017; 31: 649-656.
[23] Ramesh C, Vinithkumar NV, Kirubagaran R. Marine pigmented bacteria: A prospective source of antibacterial compounds. J Nat Sc Biol Med 2019; 10:104-113.
[24] Venil CK, Dufossé L, Devi PR. Bacterial pigments: sustainable compounds with market potential for pharma and food industry. Front Sustain Food Syst 2020; 4:100.
[25] Durán M, Faljoni-Alario A, Durán N. *Chromobacterium violaceum* and its important metabolites – review. Folia Microbiol 2010; 55: 535-547.
[26] Kothari V, Sharma S, Padia D. Recent research advances on *Chromobacterium violaceum*. Asian Pacific J Trop Med 2017; 10: 744-752.
[27] Numan M, Bashir S, Mumtaz R, Tayyab S, Rehman NU, Khan AL, et al. Therapeutic applications of bacterial pigments: a review of current status and future opportunities. Biotech 2018; 8: 207.
[28] Haun M, Pereira MF, Hoffman ME, Joyas A, Campos V, Filardi LD, et al. Bacterial chemistry. VI. Biological activities and cytotoxicity of 1,3-dihydro-2H-indol-2-one derivatives. Biol Res 1992; 25: 21.
[29] Melo PS, Maria SS, Vidal BC, Haun M, Durán N. Violacein cytotoxicity and induction of apoptosis in V79 cells. In Vitro Cell Dev Biol Anim 2000; 36: 539-543.
[30] Bromberg N, Justo GZ, Haun M, Durán N, Ferreira CV. Violacein cytotoxicity on human blood lymphocytes and effect on phosphatases. J Enzyme Inhib Med Chem 2005; 20: 449-454.
[31] Leal AM, de Queiroz JD, de Medeiros SR, Lima TK, Agnez-Lima LF. Violacein induces cell death by triggering mitochondrial membrane hyperpolarization *in vitro*. BMC Microbiol 2015;15: 115.
[32] Pereira RS, Durán N, Volpe PLO. The use of violacein to study biochemical behaviour of Saccharomyces cerevisiee cells. Eur J Drug Metab Pharmacokinet 2005; 30: 225.
[33] Cauz ACG. Carretero GPB, Saraiva GKV, Park P, Mortara L, Cuccovia IM, et al. Violacein Targets the Cytoplasmic Membrane of Bacteria. ACS Infect. Dis. 2019; 5: 539−549.
[34] Andrighetti-Frohner CR, Ktatz JM, Antonio RV, Creczynski-Pasa TB, Barardi CRM, Simoes CMO. In vitro testing for genotoxicity of violacein assessed by Comet and Micronucleus assays. Mutation Res 2006; 603: 97.
[35] Bromberg N, Dreyfuss JL, Regatieri CV, Palladino MV, Durán, N, Nader HB, et al. Growth inhibition and pro-apoptotic activity of violacein in Ehrlich ascites tumor. Chem Biol Interact 2010; 186: 43-52.
[36] Pauer H, Hardoim CCP, Teixeira FL, Miranda KR, Barbirato DS, Pires de Carvalho DP, et al. Impact of violacein from *Chromobacterium violaceum* on the mammalian gut microbiome. Plos One 2018; 13: e0203748.
[37] Antonisamy P, Ignacimuthu S. Immunomodulatory, analgesic and antipyretic effects of violacein isolated from Chromobacterium violaceum. Phytomedicine 2010; 17: 300–304.

[38] Pang L, Antonisamy P, Esmail GA, Alzeer AF, Al-Dhabi NA, Arasu MV, et al. Nephroprotective effect of pigmented violacein isolated from *Chromobacterium violaceum* in wistar rats. Saudi J Biol Sci 2020; 27: 3307-3312.

[39] Verinaud L, Lopes SCP, Prado ICN, Zanucoli F, da Costa TA, Di Gangi R, et al. Violacein Treatment Modulates Acute and Chronic Inflammation through the Suppression of Cytokine Production and Induction of Regulatory T Cells. PLoS ONE 2015; 10: e0125409.

[40] May G, Lenk W, Ott H. Trans-hydroxyviolacein, process for preparing it pure and its use for the prophylaxis and therapy of viral diseases. Germany Patent. 1989; DE3813465 (A1).

[41] May G, Brummer B, Ott H. Treatment of prophylaxis of polio and herpes virus infections - comprise administration of 3-(di:hydro-5-(hydroxy-1H- indolyl-2-oxo-3H-pyrrolidene)-di:hydro-2H-indole. Germany Patent. 1991; DE 3935066.

[42] Andrighetti-Frohner CR, Antonio RV, Creczynski-Pasa TB, Barandi CRM, Simoes CMO. Cytotoxicity and potential antiviral evaluation of violacein produced by *Chromobacterium violaceum.* Mem Inst Oswaldo Cruz 2003; 98: 834–848.

[43] Durán N, De Azevedo MBM, Alderete J. Formulation process of cyclodextrin/violacein for using as antibacterial, antitumoral, antiviral and trypanocide. Brazil Patent 1998; PIBr 9801307.

[44] Alves OL, Gimenez IF, De Azevedo MMM, Durán N, Melo PS. Pharmacological use of cyclodextrine-Au-thiol-derivative/hydrophobic compound nanoparticles as antitumoral, antibacterial, antiviral and/or antiparasites, its obtention process and formulation. Brazil Pat 2005; PIBr 0502657-1.

[45] Kallmayer V, Lanzendoerfer G, Meiring U, Mocigemba N, Reidel H, Schaefer J, et al. Cosmetic preparation, useful e.g. for the protection of skin and (semi)mucous membrane against bacteria and/or virus, comprises violacein dye in combination with lipophilic and/or hydrophilic substances. Germany Patent 2005; DE102005051869 A1.

[46] Meiring U, Lanzendoerfer G, Riedel H, Kallmayer V, Viala S, Mocigemba N, et al. Cosmetic preparation, useful e.g. for the protection of skin and (semi)mucous membrane against bacteria and/or virus, comprises violacein dye in combination with lipophilic and/or hydrophilic substances. German Patent. 2007; DE102005051869 (A1).

[47] Minbiole KPC, Harris R. Probiotic composition(s) and/or process(es) thereof. 2011; WO2011003062A3.

[48] Martin PAW, Gundersen-Rindal D, Blackburn M, Buyer J. *Chromobacterium subtsugae* sp. nov., a betaproteobacterium toxic to Colorado potato beetle and other insect pests. Int J Syst Evol Microbiol 2007;57:993-999.

[49] Blackburn MB, Sparks ME, Gundersen-Rindal DE. The genome of the insecticidal *Chromobacterium subtsugae* PRAA4-1 and its comparison with that of *Chromobacterium violaceum* ATCC 12472. Genom Data 2016;10:1–3.

[50] Farrar RR, Gundersen-Rindal DE, Kuhar D, Blackburn MB. Insecticidal activity of *Chromobacterium vaccinii.* J Entomol Sci 2018; 53: 339-346.

[51] Martin PA, Soby S. Insecticidal strains of *Chromobacterium vaccinii* sp. nov. for control of insects. USA Patent 2016; US 9339039 B1.

[52] Vöing K, Harrison A, Soby SD. Draft genome sequence of *Chromobacterium vaccinii*, a potential biocontrol agent against mosquito (Aedes aegypti) larvae. Genome Announc 2015; 3: e00477-15.

[53] Asolkar R, Huang H, Koivunen M, Marrone P. *Chromobacterium* bioactive compositions and metabolites. US Patent 2014; US8715754.

[54] Asolkar R, Huang H, Koivunen M, Marrone P. *Chromobacterium* bioactive compositions and metabolites. US Patent 2016; US 20160095323 A1.

[55] Tai W, He L, Zhang X, Pu J, Voronin D, Jiang S, et al. Characterization of the receptor-binding domain (RBD) of 2019 novel coronavirus: implication for development of RBD protein as a viral attachment inhibitor and vaccine. Cel Mol Immunol 2020; 17: 613-620.

[56] Li S, Li S, Disoma C, Zheng R, Zhou M, Razzaq A, et al. SARS-CoV-2: Mechanism of infection and emerging technologies for future prospects. Rev Med Virol 2020; 2020: e2168.

[57] Qiao B, de la Cruz MO. Enhanced binding of SARS-CoV-2 spike protein to receptor by distal polybasic cleavage sites. ACS Nano 2020; 14: 10616-10623.

[58] Jin Z, Du X, Xu Y, Deng Y, Liu M, Zhao Y, et al. Structure of M^{pro} from COVID-19 virus and discovery of its inhibitors. Nature 2020; 582: 289-293.

[59] Antony F, Vashi Y, Morla S. Therapeutic potential of nitazoxanide against Newcastle disease virus: A possible modulation of host cytokines. Cytokine 2020; 131: 155115.

[60] Rossignol JF. Nitazoxanide, a new drug candidate for the treatment of Middle East respiratory syndrome coronavirus. J Infect Public Heal 2016; 9: 227-230.

[61] Rocco PRM, Silva PL, Cruz FF, Junior MACM, Tierno PFGMM, Moura MA, et al. Early use of nitazoxanide in mild Covid-19 disease: randomised, placebo-controlled trial. Eur Respir J. 2021; doi: https://doi.org/10.1183/13993003.03725-2020).

[62] Tabata C, Tabata R, Takahashi Y, Nakamura K, Nakano T. Thalidomide prevents cigarette smoke extract induced lung damage in mice. Inter Immunopharmacol 2015; 25: 511-517.

[63] Chen C, Qi F, Shi K, Li Y, Li J, Chen Y, et al. Thalidomide combined with low-dose glucocorticoid in the treatment of COVID-19 Pneumonia. Preprints (www.preprints.org).| Posted: 26 February 2020.

[64] Caly L, Druce JD, Catton MG, Jans, DA, Wagstaff KM. The FDA-approved drug ivermectin inhibits the replication of SARS-CoV-2 in vitro. Antiviral Res 2020; 178: 104787.

[65] Kaur H, Shekhar N, Sharma S, Sarma P, Prakash A, Medhi B. Ivermectin as a potential drug for treatment of COVID-19: an in-sync review with clinical and computational attributes. Pharmacol Rep 2021; https://doi.org/10.1007/s43440-020-00195-y.

[66] Pandi-Perumal SR, Cardinali DP, Reiter RJ, Brown GM. Low melatonin as a contributor to SARS-CoV-2 disease. Melatonin Res 2020, 3: 558-576.

[67] Fernandes PA, Kinker GS, Navarro BV, Jardim VC, Ribeiro-Paz ED, Córdoba-Moreno MO, et al. Melatonin-Index as a biomarker for predicting the distribution of presymptomatic and asymptomatic SARS-CoV-2 carriers. Melatonin Res 2021; 4: 189-205.

[68] Martins D, Costa FTM, Brocchi M, Durán N. Evaluation of the antibacterial activity of poly-(D,L-lactide-co-glycolide) nanoparticles containing violacein. J Nanopart Res 2011; 13: 355-363.

[69] Queiroz KCS, Milani R, Ruela-de-Sousa RF, Fuhler GM., Justo GZ, Zambuzzi SF, et al. Violacein induces death of resistant leukaemia cells via kinome reprogramming, endoplasmic reticulum stress and golgi apparatus collapse. PLoS ONE 2012; 7: e45362.

[70] Platt D, Amara S, Mehta T, Vercuyssee K, Myles EL, Johnson G, et al. Violacein inhibits matrix metalloproteinase mediated CXCR4 expression: Potential anti-tumor effect in cancer invasion and metastasis. Biochem Biophys Res Commun 2014; 455: 107-112.

[71] Meshram RJ, Bhiodaade NH, Gacche RN, Jamgle SN. Virtual screening and docking exploration on estrogen receptors: An in silico approach to decipher novel anticancer agents. Indian J Biotechmol 2012; 11: 389-395.