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Photodynamic Therapy in the Treatment of Herpes Simplex Type 1 Virus Infection

Terapia Fotodinâmica no Tratamento de Infecção pelo Vírus Herpes Simples Tipo 1

Juliana Borges de Lima Dantas*[ab]; Marcelo Victor Coelho Marques[c]; Tila Fortuna Costa Freire[a]; Ana Carla Barletta Sanches[a]; Alena Ribeiro Alves Peixoto Medrado[d]; Gabriela Botelho Martins[e]

[a]Universidade Federal da Bahia, Institute of Health Sciences, Stricto Sensu Graduate Program in Interactive Processes of Organs and Systems. BA, Brazil.
[b]Faculdade Adventista da Bahia. BA, Brazil.
[c]Universidade Federal da Bahia, Dentistry Course. BA, Brazil.
[d]Escola Estadual de Medicina e Saúde Pública da Bahia, Dentistry Course. BA, Brazil.
[e]Universidade Federal da Bahia, Institute of Health Sciences. BA, Brazil.
*E-mail: julianadantas.pos@bahiana.edu.br

Abstract

Herpes simplex virus type 1 (HSV-1) is primarily responsible for the development of painful mucocutaneous viral lesions in the head and neck region. Antimicrobial photodynamic therapy (aPDT) consists of the use of a photosensitizing chemical substance, which interacts with an appropriate light source under the presence of oxygen, with consequent destruction or microorganisms' inactivation . The aim of this study was to conduct a literature review on the use of aPDT in the treatment of HSV-1, as well as to identify and characterize the main photosensitizing agents used in this technique. This was a narrative literature review, based on the research of scientific articles carried out in the PubMed database, from February to June 2021, using the crossing of the descriptors Decs/Mesh "photodynamic therapy" and "viral infection"; "photodynamic therapy" and "herpes virus". According to the established criteria, a total of 27 articles published in the last 20 years were included. The results demonstrate that despite the scarcity of studies involving aPDT in herpetic lesions, a single application of this therapy and with different protocols improved the clinical appearance and associated symptoms. Thus, antiviral PDT has been shown to be effective in in vitro and in vivo studies, regardless of the dye used. However, more controlled clinical trials need to be carried out in order to establish the real effectiveness of this therapeutic resource in viral infections.

Keywords: Photochemotherapy. Low-Level Light Therapy. Herpes Simplex. Herpes Labialis.

Resumo

O vírus herpes simples tipo 1 (HSV-1) é o principal responsável pelo desenvolvimento de lesões virais dolorosas mucocutâneas em região de cabeça e pescoço. A terapia fotodinâmica antimicrobiana (aPDT), por sua vez, consiste no uso de uma substância química fotossensibilizadora, que interage com uma fonte de luz apropriada sob a presença de oxigênio, com consequente destruição ou inativação de microrganismos. O objetivo do presente trabalho foi realizar uma revisão de literatura sobre o uso da aPDT no tratamento de HSV-1, bem como identificar e caracterizar os principais agentes fotossensibilizadores utilizados nessa técnica. Tratou-se de revisão narrativa de literatura, com base na pesquisa de artigos científicos realizada na base de dados *PubMed,* de fevereiro a junho de 2021, utilizando o cruzamento dos descritores Decs/Mesh *"photodinamic therapy" and "viral infection"; "photodynamic therapy" and "herpes virus".* De acordo com os critérios estabelecidos, um total de 27 artigos publicados nos últimos 20 anos foram incluídos. Os resultados demonstram que apesar da escassez de estudos que envolvam a aPDT em lesões herpéticas, uma única aplicação desta terapia e com diferentes protocolos promoveu melhora do aspecto clínico e dos sintomas associados. Desta maneira, a PDT antiviral demonstrou ser efetiva em estudos *in vitro* e *in vivo*, independente do corante adotado. Entretanto, mais ensaios clínicos controlados precisam ser realizados com o objetivo de se estabelecer a real eficácia deste recurso terapêutico em infecções virais.

Palavras-chave: Fotoquimioterapia. Terapia com Luz de Baixa Intensidade. Herpes Simples. Herpes Labial.

1 Introduction

Herpes simplex is a common viral infection in humans, and its etiologic agent is herpes simplex virus (HSV), which can be classified as herpes simplex type 1 (HSV-1) and type 2 (HSV-2). Both are double-chain DNA encapsulated viruses belonging to the *Herpesviridae* family, which present neurotropic action and rapid replication cycle. HSV-1 is the main responsible for the primary manifestation of herpetic gingivostomatitis and for the secondary development of mucocutaneous lesions in the lip region, known as herpes labialis, besides affecting nearby regions, such as oropharynx, face and eyes. HSV-2, in turn, has a preference for genital regions. Although these viruses are usually transmitted by different pathways and affect most of the time different parts of the body, the clinical manifestations of both are similar[1,2].

The transmission of the HSV-1 virus occurs through direct contact with infected secretions, and is characterized by an acute phase in which the virus rapidly replicates at the infection site, followed by the migration of this agent to the trigeminal ganglia, where it remains in an latency state[1,3,4]. Its recurrent clinical course is due to the permanence of the microorganism in the body and activation occurs in situations where the host immunity is decreased[5,6]. Although this infection does not promote life risk, its manifestation results

in unpleasant mucocutaneous symptoms, with recurrent episodes of discomfort, in which patients report tingling, pruritus, burning and pain, in addition to esthetic and social involvement[1,5,7,8].

The therapeutic options available for the management of HSV-1 infections aim to alleviate symptoms and to postpone recurrences. Conventional treatment consists of the prescription of topical and/or systemic antiviral compounds similar to nucleotides, such as acyclovir, fanciclovir and pencyclovir[7,9]. These drugs have the ability to decrease the severity of the clinical manifestation and the number of days on which the virus can be transmitted. In addition, they speed up repair and decrease the associated painful symptomatology[9]. However, studies have demonstrated that the continuous use of these drugs may promote a decrease in their efficacy, since their recurrent use may increase the possibility of developing drug-resistant viral strains due to mutations in the thymidine kinase viral[3] gene[10,11]. Due to the increasing prevalence of such viral resistance, the development of new strategies to combat or minimize the harmful effect of HSV should be carried out; however, the creation of new effective compounds is still a challenge for the pharmacological industry and scientific community[3].

Photodynamic therapy (PDT) represents a potentially effective method for promoting antimicrobial and antineoplastic action. This technique consists of the use of a chemical substance with non-toxic photosensitizing properties in biological tissues, which interacts with a light source with an appropriate wavelength; under the presence of oxygen, photochemical activation of photosensitizing promotes the formation of reactive oxygen species (EROs), which will promote the destruction or inactivation of microorganisms[3,6,12,13]. This technique is considered minimally invasive, atraumatic, well tolerated by the organism and with reduced probability of microbial resistance, besides the possibility of being used in a concomitant way with other therapeutic modalities. Recent studies have demonstrated the efficacy of aPDT in inactivation of herpes simplex virus *in vitro* and *in vivo*[2,3,6,8,14].

It is worth pointing out that the literature is not copious on this subject and that there is no narrative review that exclusively addresses the application of aPDT in HSV-1. Thus, it becomes relevant to perform the present study, since it will facilitate understanding of the mechanisms of action of this therapy on herpes virus, as well as compiling the protocols used in the recently published studies. Therefore, the objective of the present study was to carry out a narrative review of the literature on the use of aPDT in the treatment of HSV-1, as well as to identify and characterize the main photosensitizing agents used in this technique.

2 Development

2.1 Methodology

This was a narrative literature review, based on the research of scientific articles carried out in the PubMed database, from February to June 2021, using the crossing of the descriptors Decs/Mesh in English “photodynamic therapy” and “viral infection”; “photodynamic therapy” and “herpes virus”.

The inclusion criteria established were: scientific articles of all categories covering the proposed theme; publications carried out over the past 20 years and written in English. The exclusion criteria used were articles that did not address or present the technique used in a clear way, and did not describe the photosensitizer used. According to the search, a total of 347 articles were found by crossing the descriptors “*photodynamic therapy*” and “*viral infection*”, and 45 articles were identified by combining the descriptors “*photodynamic therapy*” and *“herpes virus*”, totaling 392 articles. Of these, 345 were excluded as a result of PDT employability in other diseases, without addressing HSV-1 infection; 10 articles were duplicated and 10 were written in other languages, totaling 365 excluded publications.

A total of 27 scientific articles were selected for the present study according to the established inclusion criteria, in which one was a pilot study in humans, six were case reports or cases series, seven carried out an experimental study *in vitro,* one has specifically addressed polymorphisms related to HSV-1 virus resistance. Two publications carried out a systematic review and nine adopted the narrative review of literature with relevant content for familiarization with the theme and its development.

2.2 Herpes Simplex Virus Type 1 (HSV-1)

The herpes simplex virus (HSV) is a microorganism that belongs to the *Herpesviridae* family*,* which is commonly found in humans, and which has the ability to promote lasting and persistent infection. Primary infection occurs on mucous surfaces where local epithelial cells were initially infected[1,15]. HSV can be subdivided into herpes simplex type 1 (HSV-1) and type 2 (HSV-2). Both share common structural characteristics, which include a double DNA chain protected by a nucleocapsid, a coat of tegument with viral proteins that are delivered to the host cell and an envelope with viral glycoproteins originating from the host membrane, it favors the binding and penetration of HSV in human cells, with consequent damage to the immune system. The fusion of viral and host membranes promotes the penetration of virus nucleocapsid and tegument proteins into the host cytoplasm until reaching the nucleus, with consequent expression of the viral genome. After this expression, mature viruses leave through the cellular secretory system. HSV blocks the individual’s response to infection and apoptosis of infected host cells, which ensures the virus’ survival. Although these two subtypes of viruses share similar characteristics, the

nucleotide identity is around 55%[3,14,15].

This virus can be transmitted through direct contact with infected secretions, such as saliva or the exudate of active lesions. More than 90% of the world population presents antibodies against HSV and 20% of individuals develop recurrent herpes infection by clinical manifestations[6,14], which may trigger symptoms of discomfort, pain and itching, associated with the eruption of emerging vesicles and erosions, with the possibility of promoting esthetic damage or even reducing the host's quality of life[8].

After primary infection with HSV-1, these viruses are transported along the axons of sensory nerves to the trigeminal nerve ganglion, where they become latent and reside throughout life. The resurgence and distribution of lesions in these individuals usually occur through multiple perioral ulcers, in addition to affecting the region of gingiva, oropharynx, face and eyes. The causes of recurrence may be associated with immunosuppression, exposure to ultraviolet rays, trauma and others. It is worth pointing out that, although HSV is a virus with low mortality capacity, immunosuppressed patients present a higher predisposition to severe complications[4,7].

According to the clinical aspect, these recurrent lesions progress through sequential stages, which includes the prodromic phase, with evolution to erythema, vesicles formation, ulcers, crusts and desquamation, followed by complete regression of the lesions. It is noteworthy that due to the continuity of these phases, some of them may present a rapid or even imperceptible clinical course, however with associated symptomatology[4,8]. Among the prodromic symptoms, pain, sensitivity, paresthesia and burning sensation are highlighted, which are manifested in more than 50% of the patients and with an average duration of 6 hours. The erythematous lesions appear immediately after the prodromic phase, with rapid transformation into vesicles. The vesicles phase is characterized mainly by high infectious potential, which rupture and form ulcers. Healing occurs within 10 days of the onset of symptoms[5,7,8].

It is important to point out that although HSV-1 infection is usually self-limited, this virus cannot be eradicated from the host body through the immune system. In the last 20 years, a wide variety of antiviral drugs has become available for the treatment of this condition in order to promote improvement in clinical conditions, as well as to operate on the reduction of associated symptoms, with consequent improvement in the quality of life of these patients[7].

2.3 Photodynamic Therapy

PDT, which comes from English *, Photodynamic Therapy,* was discovered more than 100 years ago, with the objective of promoting therapeutic action in specific types of cancer. The first studies focusing on antiviral activity occurred in the decade of 70, which demonstrated the ability to eliminate microorganisms under the combination of dye, appropriate light and oxygen. However, although it is not considered a new therapy, research has been carried out only in recent years, due to the progressive increase in microbial resistance to several drugs[16].

According to the literature, there are two types of PDT. The first is the antineoplastic PDT, which, as its name already suggests, aims at the treatment of malignant neoplasias through the use of a light source associated with an appropriate photosensitizer, with emphasis on hematoporphyrin derivatives. The second type of PDT, aPDT, also known as "photodynamic inactivation", "photodynamic effect", "antimicrobial photodynamic therapy", "photoactivated disinfection", "lethal photosensitization" or "photodynamic antimicrobial chemotherapy" is based on the interaction of an extrinsic photosensitizer agent associated with an appropriate light source under an oxygenated tissue, with consequent microbial inactivation through photo-oxidation, where the photosensitizer is a specific dye that operates in a lethal manner in several microorganisms, such as viruses, bacteria and fungi[17-19].

This therapy has already been investigated in detail, however, its mechanism of action is not completely understood. The initial principle of this technique involves the delivery of visible light at the appropriate wavelength associated with the photosensitizer with the objective of exciting molecules to its singlet state[12,13,20]. Several light sources can be used, however, according to the literature, the low-power laser (LBP) in the red wavelength is the one with the highest employability, since it has monochromatic light[2,8,17,20-22]. LBP has several beneficial effects, including modulation of tissue repair, inflammation process and analgesia. Because it does not cause an increase in temperature in the tissue in which it is applied, it does not have associated antimicrobial action. Thus, the association of a specific light source with an extrinsic photosensitizer agent in contact with oxygen will produce EROs, as in the case of singlet oxygen, with consequent damage to the membrane, mitochondria, nucleic acids, proteins and lipids of these microorganisms, what causes microbial death[13,15].

Its photochemical process involves the excitation of the photosensitizer molecules by the maximum absorption of a visible light photon with a wavelength that coincides with the dye absorption band, transforming it into useful energy, with consequent release of short half-life singlet oxygen[13-16]. These excited molecules are converted to the singlet state, with possible conversion through an electronic transition, called long-lasting excited triplet state, which reacts through electron transfer (type I) and/or energy transfer (type II), with radical production, and consequent cytotoxicity and microbial destruction[13,16]. The radicals produced by the two pathways are EROs, which can damage almost all types of biomolecules. The efficacy of this therapy depends on some factors, which may highlight the pH of the target tissue and the characteristics of the local cells altered, as well as the absorption capacity and the dye concentration, the light source used and the application

time, in addition to the radicals generation capacity[13-15,17].

The new concept of microbial death through aPDT has received approval in several countries, that is, it has become the basis of a new therapeutic modality today. Although its viral inactivation mechanism is not completely elucidated, it is suggested that an irreversible reaction occurs between the virus and the dye[20], with direct action on external viral structures, which includes the proteins of the capsid, lipid envelope and nucleic acids[19]. It is important to emphasize that, in order to make the virus sensitization efficient, the pre-irradiation time, which comprises the time when the dye binds to the micro-organism, must be respected. According to previous studies, this time can vary from 5 minutes[2,17,20-22] to 3 to 4 hours[8,23]. Another highlight for the photodynamic mechanism in viral infections is the proper interaction between the light source and the photosensitizer. For the light source, the type of light used and the parameters, which include power, fluency, dose per point, total dose and time of application, should be taken into account. Regarding the type of light, the red LBP plays a prominent role in research involving virus inactivation[2,8,17,18,20-22].

According to the literature, the use of aPDT in HSV-1 infections is mainly presented through *in vitro* studies[3,14,15] or case report and case series[2,17,20-22]. Currently, clinical trials with this purpose have been developed in an attempt to obtain more conclusive answers about aPDT in herpetic lesions[24,25]. The clinical applicability of this therapy in HSV-1 infections should occur in the vesicle phase, with the objective of reducing the viral load present in these cases and reducing the clinical course[2,17,20-22]. According to the PDT photochemical principle, only viral structures close to the activated dye are expected to be affected [15].

The treatment of oral HSV-1 manifestations through this phototherapeutic modality can be considered a promising alternative when used in the vesicle phase[18,20]. Among the various advantages, it can be emphasized that it is a safe, atraumatic, minimally invasive technique, and does not make use of harmful antimicrobial on adjacent tissues[20]. In addition, it requires only one clinical session and does not require the patient to perform the therapeutic regimen at home, since conventional treatments require regular use of antivirals, which may be difficult for some groups of patients, especially children[7]. Finally, it should be emphasized that this therapy works positively in cases of microbial resistance proven by the use of systemic drugs, in addition to the possibility of being used in conjunction with other therapeutic modalities, and that it presents a minimum probability of resistance against these microorganisms[13].

One of the limiting factors of aPDT is due to the fact that the delivery of light is a localized process, that is, the technique is limited to areas of the body where light can be delivered relatively easily, such as skin and exposed body cavities, which limits its field of action. In contrast, in antineoplastic PDT, the photosensitizer can be injected into the blood stream in order to accumulate in the tumor area[16,26].

2.4 In *vitro* parameters of aPDT in HSV-1

In vitro studies have demonstrated a beneficial action of antiviral PDT in HSV-1 infections, a fact that has served as a stimulus for new in *vivo* studies. Table 1 lists the aPDT protocols used for the treatment of HSV-1 in cell cultures, according to chronological order.

Table 1 - *In vitro* aPDT protocols in chronological order for the treatment of HSV-1 virus infection

Author Country	Dye	Visible light	Frequency	Results
Ayala *et al*.[27] Italy	5-ALA (Aminolaevulinic acid, Sigma Chemical Co., St Louis, EUA) for 3h.	Slide projector (Diafocus 1500-E; Kindermann & Co., Ochsenfurt, Germany), associated with tungsten lamp, wavelength ranging from 400 - 700 nm (peak 580 nm), 20 mW, energy density 18 J/cm^2 and 15 min of application.	Single application at a distance of 20 cm from the experiments.	Treatment with ALA-PDT in human keratinocytes promoted a reduction in 70% of the HSV-1 virus.
Latief *et al*.[3] China	Porphyrin TONS 504 (Okayama, Japan) diluted in distilled water, with a concentration ranging from 0.01 to 10 mg/L.	660 nm LED light (CCS, Kyoto, Japan), 0.05 W power, 10, 20 and 30 J/cm 2 power density and 3-minute application time.	One application in each energy density (10, 20 and 30 J/cm^2).	The association of LED light with different energy densities associated with porphyrin 1 mg/L promoted the inactivation of HSV-1.
Zverev *et al*.[6] England	Photoditazine (Vetagrand, Russia), derived from chlorin, at a concentration of 0, 10, 50, 100 and 200 µg/mL dissolved in 0.9% saline solution	Laser light (Polironic Corporation, Russia), 662 nm, 180 mW power, spot size 2 cm, energy doses (0.285; 0,57; 1,8; 3,42 and 10.62 J/cm^2), irradiation time 5, 10, 30 sec; 1 and 3 minutes.	Single application in two moments: the first, just after the association with the dye and HSV-1; the second, 30 minutes after the association.	The ideal antiviral effect on HSV-1 was the concentration of photosensitizer of 10 µg/mL associated with 1.8 J/cm^2, irradiation time of 30 seconds with less than 30 minutes of waiting.

Author Country	Dye	Visible light	Frequency	Results
Azizi Jalilian[14] The Netherlands	Indocyanine green 0.1 mg/mL for 5 minutes.	Infrared diode laser, 810 nm (Wuhan Gigaa Optronics Technology Co., Wuhan, China) and 940 nm (Biolase, USA), power 500 mW, *spot size* 0.384 cm^2, power density 78 J/cm^2 and 60 seconds of application.	Single application with fiber optic tip at a distance of 1 cm from the specimen.	The PDT 810 and 940nm groups associated with the dye showed a significant reduction in HSV-1 in relation to the control group (p p <0.001).
Monjo *et al*.[15] Switzerland	Botanical extract of Ortho-benzoquinone TM (Nova Scotia, Canada) at 0,05 and 0,1 μg/mL.	Red LED, 65 W of power, 12.6 J/cm^2 and irradiation time of 10 minutes.	Single application with fiber optic tip at a distance of 20 cm from the specimen.	Therapeutic effectiveness against HSV-1.

Source: Resource data.

Ayala *et al.*, in 2008[27], demonstrated by in *vitro* experimental study with human keratinocytes the ability to reduce HSV-1 by 70% by applying 5-ALA dye (Aminolaevulinic acid, Sigma Chemical Co., St Louis, USA) for 3 hours, followed by 20-cm illumination away with slide projector (Diafocus 1500-E; Kindermann & Co., Ochsenfurt, Germany), associated with a tungsten lamp with a wavelength of 580 nm peak (range from 400 to 700 nm), 20 mW, energy density of 18 J/cm^2 and 15 min application in the specimen with the virus. 5-ALA dye represents the first compound in the porphyrins synthesis pathway, which is widely used in dermatology for the treatment of cutaneous malignant neoplasms.

In *vitro* study by Latief *et al.*[3], the efficacy of antiviral PDT with dye derived from porphyrin, TONS 504 (Okayama, Japan) diluted in distilled water, was evaluated to obtain differentiated concentrations ranging from 0.01 to 10 mg/L. To photoactivate this dye, an LED light (Light Emitting Diode) with a wavelength of 660 nm, a power of 0.055 W and irradiation time of 3 minutes was used. Different energy densities were adopted in order to inactivate HSV-1. The results showed that the association of LED light with energy density between 10 and 30 J/cm^2 with concentrated porphyrin at 1 mg/L promoted viral inactivation. It is worth pointing out that this dye is widely used for the treatment of malignant neoplasms associated with phototherapy, but in the present study it has also shown beneficial effect through aPDT.

In the experiment carried out by Zverev *et al.*[6], the effect of PDT on the treatment of HSV infection was examined using an *in vitro* model. For this purpose, second generation chlorine e_6 photosensitizer, called Photoditazine (Vetagrand, Russia), was used at a concentration of 10 μg/mL, which is derived from porphyrin and belonging to the class of tetrapyrrolic substances, which has strong absorption in the red wavelength. The light source adopted was laser light with a wavelength of 662 nm, power of 180 mW, *spot size* of 2 cm, power density of 1.8 J/cm^2 and total irradiation time of 30 seconds. According to the results, PDT was capable of promoting antiviral effect in the in *vitro* model with cell culture of HSV-1 virus, which demonstrates positive results of second generation dye in antimicrobial treatment, corroborating previous results by Latief *et al.*[3].

In a study conducted by Monjo *et al.*[15], orthoquinone at 0.05 and 0.1 μg/mL (Nova Scotia, Canada) was selected as a photosensitizer agent for HSV-1 infection treatment, due to its antimicrobial and antioxidant properties s that come from the natural extracts present in this medicinal plant, which includes resveratrol and emodin. To photoactivate this compound, a red LED light was used, with 65 W of power, 12.6 J/cm^2 and irradiation time of 10 minutes at a distance of 20 cm from the sample. The results indicated that the use of this agent associated with LED light demonstrated therapeutic effectiveness against HSV-1, in which the authors suggest that orthoquinone may be a viable alternative in cases of viral infections resistant to current drugs, however, they emphasize the need for more studies that relate this agent to phototherapy.

In a recent *in vitro* study by Azizi Jalilian[14] , APDT with infrared wavelength in HSV-1 infection was used. Diode laser was introduced at wavelengths 810 nm (Wuhan Gigaa Optronics Technology Co., Wuhan, China) and 940 nm (Biolase, USA) with power of 500 mW, energy density of 78 J/cm^2 associated with the previous application for 5 minutes of green indocyanine dye at 0.1 mg/mL (Periogreen, Elixxion, AG Radolfzell, Germany). Green indocyanine is a water-soluble fluorescent photosensitizer agent, commonly used in endoscopy procedures and to test liver function through its intravenous infusion. This dye shows a high light absorption capacity in the wavelength ranging from 600 to 800 nm, however, its maximum absorption occurs at around 800 to 805 nm. The laser light application time was 60 seconds. Both wavelengths associated with green indocyanine showed superior results in relation to the control group (p <0.001). When comparing the different wavelengths between each other, it can be observed that the irradiation with 810 nm diode laser associated with the dye promoted a

higher quantitative reduction of HSV-1 compared to the 940 nm group ($p < 0.001$), fact which reaffirms a more effective action of this agent under irradiation at a wavelength close to 805 nm.

2.5 Clinical parameters of aPDT in HSV-1

The current literature lacks clinical studies that address the direct action of PDT in HSV-1 infections, however, despite the lack of research in this area, the results are promising. Among studies with human beings, the populations studied involve few participants[8] and are more comprehensive for case reports or cases series[2,17,20-22]. From the search performed, only one clinical trial completed to the present moment was found[25] and one is in execution phase[24] (Table 2).

Table 2 - Clinical protocols of aPDT in chronological order for the treatment of HSV-1 virus infection

Author/Country Type of study	Dye	Visible light	Frequency	Results
Sperandio et al.20 Brazil Clinical case	Methylene blue 0.01% for 5 minutes.	Red laser 660 nm (Photon Lase III, DMC®), power 100 mW, power density 100 J/cm^2, application of 28 seconds per point, with 2.8 J of power at each point (total 8.4 J) and spot size 0.028 cm^2.	Single application in the vesicle phase. 3 lesion points were selected for application.	The two clinical cases presented had complete remission between 48 and 72 hours after, with absence of recurrence in the first 6 months of follow-up.
Marotti *et* al.17 Brazil Clinical case	Methylene blue 0.01% for 5 minutes.	Red laser 660 nm (Twin Laser, MM Optics®, São Carlos, Brazil), power 40 mW, Energy density 120 J/cm^2, application of 2 minutes per point, with 4.8 J of energy at each point (total of 9.2 J) and *spot size* of 0.04 cm^2.	Single application in the vesicle phase. 4 lesion points were selected for application.	The four clinical cases presented had complete remission on the 7th day after application, with recurrence of only 1 case before completing the first 6 months of follow-up.
Marotti et al.21 Brazil Clinical case	Methylene blue 0.01% for 5 minutes.	Red laser 660 nm (Photon Lase III, DMC®), power 100 mW, power density 100 J/cm^2, application of 28 seconds per point, with 2.8 J of power at each point (total 8.4 J) and spot size 0.028 cm^2.	Single application in the vesicle phase. 3 (case 1) and 5 points (case 2) of the lesion were selected to perform the application.	The two clinical cases presented had complete remission within 7 days, with absence of recurrence in the first 6 months of follow-up.
Ramalho et al.22 Brazil Clinical case	Methylene blue 0.05% for 5 minutes.	660 nm red diode laser (MM Optics®, São Carlos, Brazil), 100 mW of power, 120 J/cm^2 of total power, 4.8 J of power per point and 2 min of irradiation.	Single application in the vesicle phase (case 1) and in the macula phase (case 2). The number of points has not been mentioned.	After 24 hours, the regression of the lesions could be observed in both patients.
Osiecka et al.8 Poland Pilot study	Aminolaevulinic acid (ALA) 20% cream. Waited 4h for irradiation.	Red laser 630 nm ± 20 nm (Penta lamps, Teclas, Switzerland), with power of 100 mW and total power density of 120 J/cm^2.	Single application in the initial phase (erythema/ vesicle). The authors did not inform the amount of points of the lesion that were irradiated.	All the 6 patients presented delayed healing. However, in the first 12 months of follow-up, they demonstrated absence of recurrence.
Lago *et* al.2 Brazil Clinical case	Methylene blue 0.01% for 3 minutes.	660 nm red laser (Laser Duo, MM Optics®, São Carlos, SP, Brazil), power 100 mW, 2 minute application per point, with 3 J of power at each point (total 12 J) and 0.04 cm *spot size*^{2}.	Single application in the vesicle phase. 4 lesion points were selected for application.	After the first 24 hours, there was a considerable reduction in signs and symptoms, with crust formation.
La Selva et al.24 Brazil Study protocol for Clinical trial	Methylene blue 0.05% for 1 minute.	660 nm red laser (Laser Duo, MM Optics®, São Carlos, SP, Brazil), power 100 mW, 300 J/cm^2, 3J for 30 sec and 3 mm^2 *spot size*.	Single application in the center of the lesion, vesicle phase.	Preliminary results have not yet been disclosed.
Ramalho et al.25 Brazil Clinical trial	Methylene blue 0.05% for 5 minutes.	660 nm red laser (Laser Duo, MM Optics®, São Carlos, SP, Brazil), power 40 mW, 120 J/cm^2, 4.8J and 120 sec application per point.	Single application in the vesicle phase. The number of points was determined by the size of the lesion, with a distance of 1 cm of application.	Only in the first 24 hours did the APDT group show superior results (pain, edema and healing) in relation to the group using the topical antiviral acyclovir (p>0.05).

Source: Resource data.

The search for more effective intervention methods in the treatment of HSV-1 motivates the research of different strategies, sometimes using the association of existing techniques with new therapeutic modalities. To this end, Ramalho *et al.*[25], performed a randomized controlled clinical trial with 75 patients with active vesicle labial herpes, comparing three different protocols based on the use of aPDT and antiviral medication (G1: PDT with methylene blue and LBP 660 nm; G2: Topical use of antiviral acyclovir 5%; G3: Association of both therapies. The variables wound size, healing time, edema and tingling were evaluated. The results showed that aPDT showed immediate advantages, still on the first day, in relation to edema and tingling sensation, with superiority in relation to the exclusive use of acyclovir. However, regarding the healing time, the three groups presented similar results. Thus, it could be concluded that aPDT demonstrated apparent superiority over acyclovir in the early stages of infection, in terms of edema and tingling sensation.

In a systematic review based on clinical trials and case reports of several viral infections, carried out by Kelley and Rashid[7], it could be observed that despite the lack of studies, the use of aPDT with neutral chlorine red, together with an ultraviolet light (UVA) of 365 nm for the treatment of HSV skin manifestations promoted the lesions clinical reduction, in addition to increasing the time interval for the virus reactivation. Another relevant information was found that this therapy promoted immunomodulatory effect by increasing IL-6 expression and CD8 T lymphocyte activity. Its role in the stimulation of the host's immune system was also approached by Dai *et al.*[16].

In a pilot study carried out by Osiecka *et al*.[8], six patients with simple labial herpes were submitted to PDT with porphyrin-derived cream (20% aminolaevulinic acid - ALA, DMSO®) at the initial stage of the lesion, represented by erythema, papules or vesicles. The light source used to activate ALA was red light with a wavelength of 630 nm ± 20 nm, from an allogeneic lamp (Penta Lamps, Teclas, Switzerland), with a power of 100 mW and a total energy density of 120 J/cm^2, which was applied after 4 hours of initial insertion of ALA. According to the authors, the results revealed that the high irradiation dosage at the initial stage of the infection caused an acute inflammatory reaction, which led to an increase in the subsequent stages of crust and reepithelization. However, all patients responded positively to the treatment employed, with no recurrence in the first 12 months of follow-up. Thus, this methodology adopted was not able to reduce the clinical course of HSV-1 infection, however, it promoted the delay in the resurgence of new lesions.

Sperandio *et al.*[20], selected 0.01% methylene blue associated with LBP for the treatment of two clinical cases of simplex herpes labialis in the vesicle phase. The proposed protocol was the prior application of the photosensitizer agent during the 5-minute period, after rupture of the vesicles with sterile needle, followed by irradiation with the red laser (660 nm). After a single application, the patients reported immediate relief in the symptomatology, and 48 to 72 hours after the initial session, there was remission of the signs and symptoms initially present. A 6-month follow-up was performed, which did not show recurrence of the lesions, as well as associated symptoms, which according to the patients' previous report occurred very frequently. In a case report by Lago *et al.*[2], methylene blue was also used at 0.01% associated with LBP at 660 nm for the treatment of patients with recurrent infection by HSV-1 in the nose region. However, the pre-irradiation time in the present case was 3 minutes, that is, the dye contact time and the onset of photoactivation was shorter compared to the study by Sperandio *et al.*[20]. The patient reported immediate relief after irradiation and after the first 24 hours, there was formation of crust, that is, probable inactivation of the virus occurred quickly and with a decrease in the initial signs and symptoms. According to the results obtained, it can be observed that the present study demonstrated remission of the signs earlier than the previous study, which obtained this same aspect in a longer period, equivalent to 48 to 72 hours after the initial session.

Marotti *et al.*[17], in their case series study, they used aPDT in 4 patients with simplex herpes labialis in vesicle phase. The criteria established for the use of the dye were the same as those adopted by Sperandio *et al.*[20], however, there was variation in photoactivation with LBP. Irradiation occurred for 8 minutes, also divided into 4 different points, with 2 minutes of application per point. The results showed that in the first 24 hours after aPDT, all the cases obtained a satisfactory tissue repair, with complete healing reach after 7 days after the beginning of treatment. Regarding recurrence rates, only 1 case showed new lesions before completing the first 6 months of follow-up. Thus, the treatment of herpes labialis with aPDT was effective, did not show side effects and, when associated with laser phototherapy, accelerated the healing process. The results obtained were similar to the case report by Lago et al.[2].

In 2010, Marotti *et al.*[21], performed aPDT associated with LBP for the management of recurrent simplex herpes labialis in the vesicle phase in two patients. First, the vesicles were drained with a sterile needle, followed by the dye application. The protocol established for both cases was the same as in previous studies carried out by Marotti *et al.*[17] and Sperandio *et al.*[20], in which 0.01% methylene blue was used for 5 minutes, followed by the application of the red laser diode with a wavelength of 660 nm. The number of points applied varied between the two cases due to the size of the lesion, where the first case had a total energy dose of 8.4 J, divided into 3 application points, while in the second case the total energy dose was 14 J, distributed at 5 points of the lesion. The crust phase was rapidly manifested, and in the first case there was crust formation after the first 24 hours, and in the second, this pattern was reached after 48 hours. The complete remission of the lesions occurred 7 days after the initial session, with

no recurrence in the 6 subsequent follow-up months. Thus, the satisfactory results of the present study corroborate studies carried out with similar protocols[17,20] and with a dissimilar protocol by Lago *et al.*[2] and Ramalho *et al.*[22].

Ramalho *et al.*[22], used methylene blue at a concentration of 0.05% as a sensitizer in two patients with recurrent lesions of simplex herpes labialis. The pre-irradiation time was 5 minutes, and after this period, the red LBP was applied at a wavelength of 660 nm. After 24 hours, the regression of the lesions could be observed in both patients, which demonstrates the effectiveness of methylene blue in different concentration than what was already covered in the literature in previous studies[17,20,21] as a photosensitizer agent in the treatment of simplex herpes labialis.

Nobbe *et al.*[23], reported an atypical case in which an elderly patient, aged 81 years, after being submitted to aPDT in the frontal face region for treatment of actinic keratosis after squamous cell carcinoma in the region, showed recurrent simplex herpes lesions 24 hours post-PDT. The protocol used included the previous use of topical photosensitizer methyl amino levulinate (Metvixs®) during the 3-hour period, followed by activation with non-coherent red light (ActiLight®). This agent is methyl-5-aminolevulinate, which is approved for the use of basal cell carcinoma through antineoplastic PDT. The possible causes for this phenomenon may be attributed to the possibility of the patient's immunosuppression at the time of application, since the latter presented a previous history of cancer in the region, together with the fact that the advanced age, which in itself, may be a predisposing risk factor. In addition, this manifestation may have occurred as a result of the adopted PDT protocol, since the parameters of the light source used were not informed.

3 Conclusion

The current literature lacks clinical trials on the application of aPDT in HSV-1 manifestations, possibly due to the difficulty recruiting patients for controlled clinical studies. However, despite the lack of work, in *vitro* and in *vivo* studies show that a single application of this therapy was able to promote antiviral action in HSV-1 infections.

Regarding the photosensitizer agents, methylene blue obtained satisfactory results in all the studies, even when used in different concentrations and with variation in pre-irradiation time. In *in vitro* studies, dyes derived from porphyrins, which present a wide use for the treatment of skin neoplasms, presented promising results in antiviral PDT. It should be noted that the chemical photosensitizer and the inherent photophysical properties of this agent, as well as the concentration used, are extremely important for the success of this therapy.

Regarding the parameters of the light source, the studies have not shown that there is a protocol that is considered gold standard, since this determination is not simple, since this therapeutic modality requires a set of factors for the success of the treatment, this includes device dosimetry such as wavelength, power, exposure time, fluency rate, energy per point, and total energy. However, despite the great variation of these protocols, as well as the different light sources used, therapeutic success could be observed.

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