Towards effective cutaneous leishmaniasis treatment with light-based technologies. A systematic review and meta-analysis of preclinical studies

Fernanda Viana Cabral [a,1], Tiago Henrique dos Santos Souza [b,1], Fábio Parra Sellera [c,d,1], Adriana Fontes [b,1], Martha Simões Ribeiro [a,1,*]

[a] Center for Lasers and Applications, Energy and Nuclear Research Institute (IPEN/CNEN), São Paulo, SP, Brazil
[b] Department of Biophysics and Radiobiology, Federal University of Pernambuco, Recife, PE, Brazil
[c] Department of Internal Medicine, School of Veterinary Medicine and Animal Science, University of São Paulo, São Paulo, SP, Brazil
[d] School of Veterinary Medicine, Metropolitan University of Santos, Santos, SP, Brazil

ARTICLE INFO

ABSTRACT

Cutaneous leishmaniasis (CL) is a neglected disease that represents a serious global public health concern. We performed a systematic review with meta-analysis targeting the use of light-based therapies on CL in preclinical studies since they are essential to identify the benefits, challenges, and limitations of proposing new technologies to fight CL. We searched Pubmed and Web of Science to include original preclinical researches in English that used light-based technologies to fight CL. Inclusion criteria encompassed any animal model for CL induction, an untreated infected group as the comparator, reliable and consistent methodology to develop and treat CL, focus on an antimicrobial therapeutic approach, and data for lesion size and/or parasite load in the infection site. We identified eight eligible articles, and all of them used photodynamic therapy (PDT). For the meta-analysis, three studies were included regarding the parasite load in the infection site and four comprised the lesion size. No overall statistically significant differences were observed between untreated control and PDT groups for parasite load. Differently, PDT significantly reduced the lesion size regardless of the protocol used to treat CL (in mm, SMD: -1.90; 95% CI: -3.74 to -0.07, $p = 0.04$). This finding is particularly encouraging since CL promotes disfiguring lesions that profoundly affect the quality of life of patients. We conclude that PDT is a new promising technology able to be topically used against CL if applied in more than one session, making it a promising ally for the management of CL.

1. Introduction

Leishmaniasis is a group of vector-borne diseases caused by protozoan parasites of the genus *Leishmania*, commonly transmitted by infected sandflies during their blood meal [1,2]. It has been targeted as one of the World Health Organization top 20 neglected tropical diseases, affecting mostly people from low- and middle-income countries that live in poor housing conditions with malnutrition, have a weak immune system, and lack basic health resources [3].

Although leishmaniasis can cause several forms of clinical manifestations, the cutaneous presentation remains the most prevalent worldwide [1,2,4]. Cutaneous leishmaniasis (CL) poses a substantial clinical challenge due to long treatment regimens, the emergence of drug-resistant parasites, and treatment failure [1,2,4]. Besides, the disfiguring lesions of active CL on exposed parts of the body for more than one year may lead to severe psychological impacts such as decreased body satisfaction, anxiety, depressive symptoms, resulting in a low quality of life for CL-affected patients [5].

There are few advances in CL treatment and no consensus on its best management. Current therapy includes the use of different drugs that have particular benefits and limitations so they are still ineffective [1,4,6]. Pentavalent antimony has been considered the first-line anti-leishmanial drug, however, the increasing reports of clinical isolates of *Leishmania* spp. resistant to this compound has restricted its usage [7]. Second-line drugs include amphotericin and pentamidine, but emerging resistance and acute toxicity have been considered limiting factors for their administration [1,7]. Miltefosine, an anticancer agent, is the only oral drug currently available. However, it has not been approved for leishmaniasis treatment in many countries, as the ease with which miltefosine-resistant strains are selected *in vitro* has been reported [8]. Moreover, the main disadvantages include teratogenicity effects and long lifespan (an essential contributing factor to drug resistance) [6,9]. Topical treatments with cryotherapy, thermotherapy, and topical paromomycin have also been explored for CL form, but with variable results [1]. Indeed, the current scenario demands an urgent search for novel approaches for CL, and light-based therapies have been in the spotlight as an interesting strategy to treat localized infections, including leishmaniasis [10,11].

Antimicrobial light-based approaches encompass the use of light alone or the association of light and photoactivated compounds to reach antimicrobial effects. Among them, antimicrobial photodynamic therapy (PDT) and antimicrobial blue light (aBL) treatment at 400–470 nm have been broadly investigated to achieve microbial inactivation [10,12,13]. PDT combines the administration of a photosensitizer (PS) agent that is activated by a low power light source, at a wavelength matching the PS absorption band, to promote photochemical reactions, leading to the generation of reactive oxygen species (ROS) [10]. On the other hand, aBL can be absorbed by endogenous pigments (*e.g.,* flavins and porphyrins), resulting in ROS production [13]. As sunlight comprises a broad range of wavelengths in ultraviolet and visible regions, this kind of irradiation has also been suggested as an antimicrobial approach [14,15]. It is well known that ROS are detrimental to microbial cells, and therefore these antimicrobial light-based therapies can inactivate a broad range of pathogens with proper light doses [10,12,13]. From this perspective, antimicrobial light-based technology has been demonstrated to have a high potential to fight infectious diseases, being also an attractive cost-effective candidate [16].

As most conventional treatments are based on long-term multiple administrations, an ideal therapy for CL should be efficient within a short-term period. Besides, it should be minimally invasive or toxic, with low potential to induce resistance. For example, it is well known that *Leishmania* parasites are highly susceptible to oxidative stress. Thus, increased levels of ROS promoted by PDT can strongly disturb their redox state, resulting in the parasite's death. Because of its immediate results, PDT might shorten the duration of treatment, contributing to patient compliance.

However, the severity of leishmaniasis depends not only on the virulence of the wide range of *Leishmania* species but also on the host immune response, which plays an essential role in either disease progression or healing [1]. Thus, animal models significantly contribute to advance in the development of new therapies against CL. Herein, we systematically revised preclinical studies of light-based technologies to fight CL.

2. Methods

We followed the protocol recommended by the SYRCLE (SYstematic Review Center for Laboratory animal Experimentation) to conduct this systematic review [17]. We specified the population, intervention, control, and outcome to identify and include comparative preclinical studies of CL models that investigated the potential of antimicrobial light-based therapies for the primary outcome of two quantitative data: lesion size and parasite load.

The search was performed using two bibliographical databases: Pubmed and Web of Science from inception until September 2020. The following search terms were used: "cutaneous leishmaniasis" AND ("photodynamic" OR "photoinactivation" OR "photosensitization" OR "phototherapy" OR "photochemotherapy" OR "light therapy" OR "blue light" OR "sunlight" OR "daylight" OR "laser" OR "light-emitting diode") to cover all modalities of light sources and/or light-based therapies to fight CL. The protocol for this review was registered on the PROSPERO (International Prospective Register of Systematic Reviews, registration number: CRD42020212365).

We included original research articles in any animal model published in English. After identification, duplicate articles were excluded. The screening was performed after reading titles and/or abstracts, which excluded reviews, clinical trials, case reports, *in vitro* assays, and studies published in languages other than English. The titles and abstracts were independently screened by all reviewers and checked for agreement.

For eligibility, studies should present consistent methodology, reliable light and/or PS parameters, infected untreated group, besides focusing on the treatment of CL. We used the infected untreated group as the control for comparison with the intervention group since light-based therapies are used topically and conventional antileishmanial drugs are systemically administered.

Articles also should contain quantitative or scored results for lesion size and/or reduction of parasite load at the site of infection. We excluded studies when the data were duplicate. Full articles tried by title and abstract were read and independently appraised by two reviewers (FVC and THSS) considering the eligibility criteria. Discrepancies were discussed by all authors.

The quality of included studies was assessed by SYRCLE's risk of bias (RoB) tool and performed by two reviewers (FVC and MSR) [18]. The degree of bias was categorized as low, high or unclear related to the following topics: 1) sequence generation, baseline characteristics, and allocation concealment (selection bias); 2) random housing and blinding (performance bias); 3) random outcome assessment and blinding (detection bias); 4) incomplete outcome data (attrition bias); 5) selective outcome reporting (reporting bias); and 6) other sources of bias. We used the Kappa coefficient to evaluate the agreement between reviewers and discrepancies were resolved by discussion among all reviewers.

For the meta-analysis, we included only the articles that presented the number of animals per group with quantitative measurements for lesion size and/or parasite load. We contacted the authors if some data was unclear. Due to expected heterogeneity among studies, we performed a random effect meta-analysis to calculate the reduction of lesion size and/or parasite load at the site of infection at the end of treatment. Mean values $\pm$ standard deviations were estimated from the graphs of the studies if not reported by authors. The effect size was assessed and reported as the standardized mean differences (SMD) between treated and untreated infected groups with 95% confidence intervals (95% CI). Weight was calculated based on the inverse of the variance. Statistical analysis was conducted by Cochrane RevMan software (Review Manager 5.4).

3. Results

Overall, 353 records were found through Database searching on Pubmed and Web of Science. No additional records were identified through any other sources. Duplicate articles were then removed, resulting in 246 publications for screening. Afterward, by reading the title and/or abstract, 223 articles were excluded. As a result, 23 full-text manuscripts were selected for eligibility of which 15 were excluded because they did not meet the inclusion criteria (Fig. 1).

Indeed, four publications were focused on vaccination and host immune response modulation [19–22], and one was focused on treatment optimization conjugating the PS to TiO_2 nanoparticles [23]. Three articles did not show reliable light parameters, *i.e.,* the combination of light dose, power density, and exposure time were conflicting [24–26]. The other four did not report enough data due to the absence of some
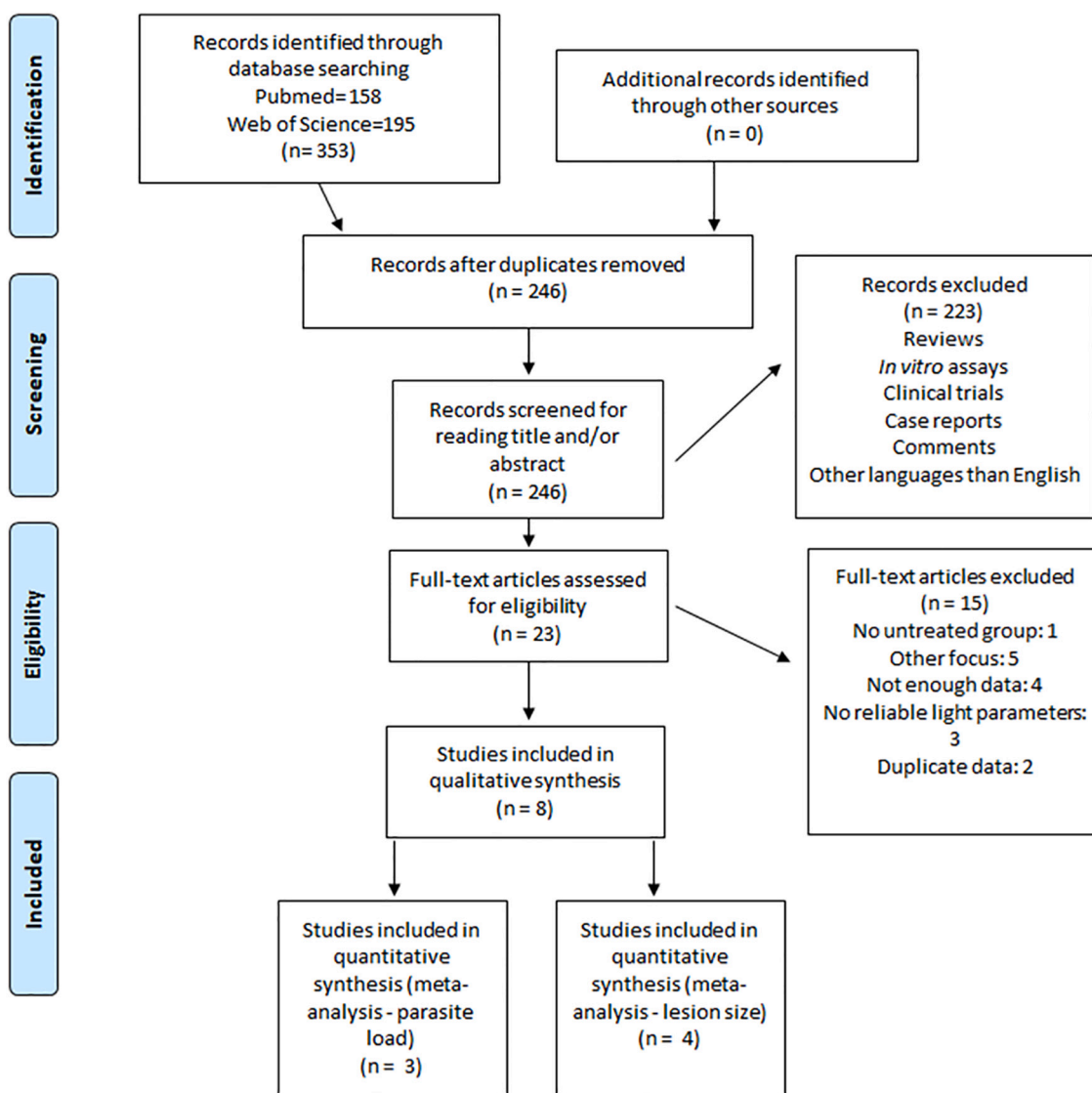
Fig. 1. Study selection flow chart according to the PRISMA guidelines.

light parameters [27–29] or the infected untreated control group [30], two showed the same experimental design for CL induction and treatment conducting to duplicate data [31,32], and the last one did not present quantitative data regarding the lesion size or the parasite load on the infection site [33]. Therefore, eight papers were included for the systematic review, and all of them used PDT to treat CL. For the meta-analysis, three studies were included regarding the parasite load in the infection site and four comprised the lesion size (Fig. 1).

We noticed a diversity of animal models for *Leishmania* species and different sites of infection. We have found four articles that performed assays using Old World *Leishmania* species (*L. major*) inoculated in the ear of BALB/c mice [34–37]. One of these works designed the experiments using L. *major* expressing green fluorescent protein (GFP+) to monitor parasitic load by measuring GFP fluorescence intensity [34] (Table 1).

New World CL species were found in the other studies, in which one of them golden hamsters were infected in the footpad with L. *braziliensis* [38]. Two studies used wild-type L. *amazonensis*, for which one induced CL in BALB/c mice in the base of the tail [39] and the other infected C57BL/6 mice in the footpad [40]. One study induced CL in the footpad

Table 1
Biological conditions used to induce CL infection in the selected studies.

Animal model	Gender	Age (days)	*Leishmania* species	Inoculation (number of parasites)	Infection site	Infection time (days)
BALB/c mice [39]	Female	35–42	*L. amazonensis* (amastigotes)	1×10^6	Base of the tail	45
Golden hamsters [38]	Female	90	*L. braziliensis* (promastigotes)	4×10^7	Right footpad	90
C57BL/6 mice [40]	Female	70	*L. amazonensis* (promastigotes)	3×10^6	Hind paw	30
BALB/c mice [41]	Female	56	*L. amazonensis* (LUC) (promastigotes)	1×10^6	Left footpad	28
BALB/c mice [34]	Female	42–56	*L. major* (GFP) (promastigotes)	1×10^6	Ear	21
BALB/c mice [35]	Female	42–56	*L. major* (promastigotes)	1×10^6	Ear	28–56
BALB/c mice [36]	female	42–56	*L. major* (promastigotes)	1×10^6	Ear	21
BALB/c mice [37]	Female	42–56	*L. major* (promastigotes)	1×10^6	Ear	21

of BALB/c mice with an L. *amazonensis* recombinant strain expressing the luciferase gene (La-LUC) to monitor in real-time the parasite burden [41] (Table 1).

We have also realized an interesting standardization in biological parameters in terms of gender and the number of parasites used to induce CL. All animals used were female. We found in five papers an average of between 6 and 8 weeks old BALB/c mice, in which animals had been infected by inoculation of 1×10^6 promastigotes (extracellular form) [34–37,41]. In another one, CL was induced by 1×10^6 amastigotes (intracellular form) in the base of the tail [39]. We found that in two papers other animal models of distinct ages were infected with different inoculum [38–40] (Table 1).

In terms of light sources and parameters, studies were more heterogeneous. All studies were addressed by using a wavelength within a range between 660 and 670 nm. Three articles used a laser [36,39,40] in contrast to the other three studies that have reported an LC-122A lamp (LumaCare) as the main light source [34,35,37]. Only two works performed red LED (light-emitting diode)-based PDT [38,41]. There was a huge variability in power density and exposure time, delivering different light doses in the infected tissue. Moreover, no standard was noticed for the PS as well. Different concentrations, formulations, routes of administration, number of sessions, and dark periods were assessed (Table 2).

The parasite load quantification was assessed at the site of infection by limiting dilution assay after animal euthanasia, except for L. *major* GFP+ and La-LUC strains that were continuously monitored after PDT by either fluorescence or bioluminescence, respectively [34,41]. Another article has performed a scored analysis at the site of infection through histological evaluation [39]. The disease progression was also evaluated by measuring lesion size in four articles, whereas in the other four, the authors did not address this issue (Table 3).

Regarding the risk of bias (Fig. 2), for the sequence generation, we noticed that only one study reported that animals were randomly divided into untreated control and PDT groups [41]. Additionally, one study reported that animals were distributed into groups according to the lesion size indicating that the baseline was not the same for the groups [39]. Another study has made no clear the time of infection when PDT was applied [35].

Allocation concealment, random and blinding housing as well as random outcome assessment were unclear in all studies since authors did not address these issues. Although we have considered that the outcome evaluation was not blinded, we have assumed a low risk of bias

for all studies, which reported score or quantitative data for parasite load and lesion size.

Selective outcome reporting was stated as a high risk of bias when we observed any selective and/or lack of information over methodology or results. Besides, studies with unpaired data regarding the number of animals per group and plotted results were considered with a high risk of bias related to attrition bias. In this context, we identified that three studies reported different numbers of data per group suggesting that control and PDT groups were unpaired [35–37]. In one study, the authors did not describe how the lesion size was measured [35].

We defined the other risks of bias as the lack of information regarding the number of animals. One article was considered with a high risk of bias since it did not mention the number of animals per group [34]. In the other four articles the number of animals for untreated control and PDT groups was unclear [35–37,40] (Fig. 2). A good agreement of 80% was achieved between reviewers (Kappa index = 0.7).

Fig. 3 presents the results of the meta-analysis comparison for lesion size (Fig. 3A) and parasite load (Fig. 3B). Four studies compared lesion size (in mm) for PDT ($n = 27$) and untreated control group (n = 27) [38–41]. We noticed a statistically significant decrease in lesion size (in mm, SMD: -1.90; 95% CI: -3.74 to -0.07, $p = 0.04$) for animals that

Table 3
Best results achieved by the selected studies for CL treatment.

Follow-up (days)	Mean values of lesion size (mm) (PDT *vs.* control)	Mean values of parasite load (PDT *vs.* control)
21 [39]	12.8 *vs.* 13.3	1×10^4 *vs.* 1×10^6 (limiting dilution)
84 [38]	2.5 *vs.* 4.0	Weak *vs.* moderate (histology)
20 [40]	2.5 *vs.* 2.7	1.2×10^5 *vs.* 1.4×10^5 (limiting dilution)
30 [41]	0.75 *vs.* 2.0[a]	4×10^7 *vs.* 2×10^8 (bioluminescence)
6 [34]	–	50 *vs.* 100% (fluorescence)
4 (1 session)	0.2 *vs.* 0.6	1×10^5 *vs.* 1×10^7
12 (3 sessions) [35]	–	5×10 *vs.* 5×10^6 (limiting dilution)
7 [36]	–	1×10 *vs.* 1×10^4 (limiting dilution)
5 (3 sessions) [37]	–	1 *vs.* 0.005 (limiting dilution)

[a] Informed by the author.

Table 2
Parameters applied by the selected studies to reach the best results in the CL treatment.

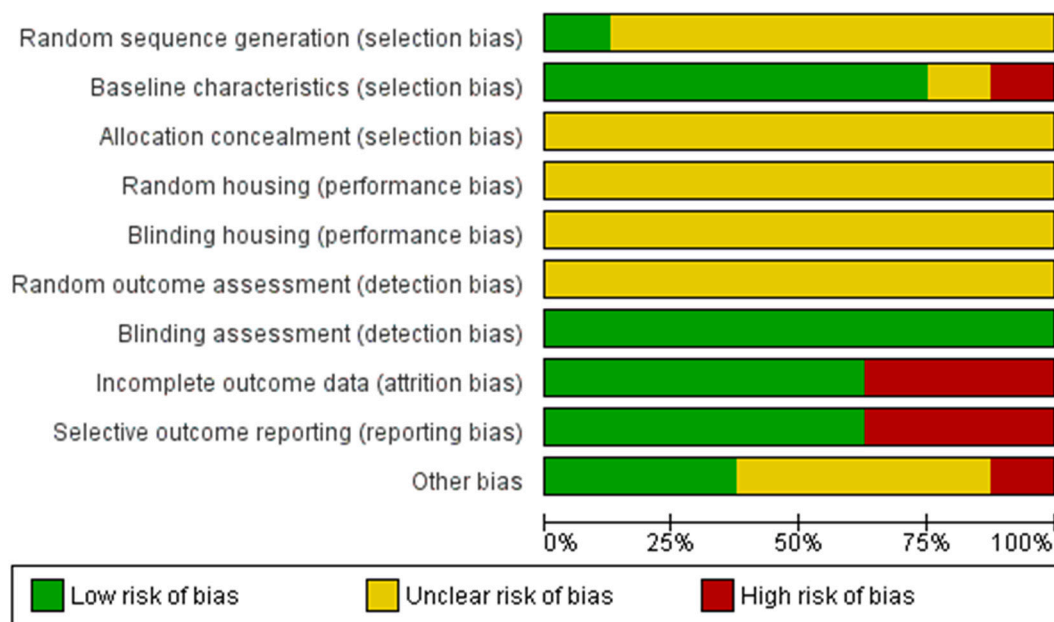| PS	Concentration	Formulation	Administration	Dark period (min)	Light source	λ (nm)	Power density (mW/cm^2)	Radiant exposure (J/cm^2)	Exposure time (s)	Sessions
AlClPC [39]	3.6 ± 0.9 μg/mL	Liposomal	Topical	15	Laser	660	81	95	1200	10
MB [38]	10 mM	Water solution	Topical	–	6 LEDs in a series	663	5/LED	18/LED[a]	3600	36
AlClPC [40]	5 μM	Gel liposomal	Topical	15	Laser	670	80	100	1250[a]	10
MB [41]	100 μM	PBS	Subcutaneous	10	LED	660 ± 11	100	150	1500	2
PPA904 [34]	2 mg/cm^2	Cream	Topical	60	LC-122A	665 ± 15	30	21	696	1
PPA904 [35]	500 μM	Cream	Topical	30 60 90 120	LC-122A	665 ± 15	50	50	1000[a]	1 and 3
PpIX [36] (ALA)	20%	Solution	Topical	240	Laser	635	66	50	~760[a]	1
PPA904 [37]	10.7 μM 500 μM	Solution Cream	Intralesional Topical	30	LC-122A	665 ± 15	60	50	~830[a]	1 3

[a] Calculated by reviewers.

Fig. 2. Risk of bias graph for the eligible studies. "Other bias" refers to the lack of information regarding the number of animals per group.
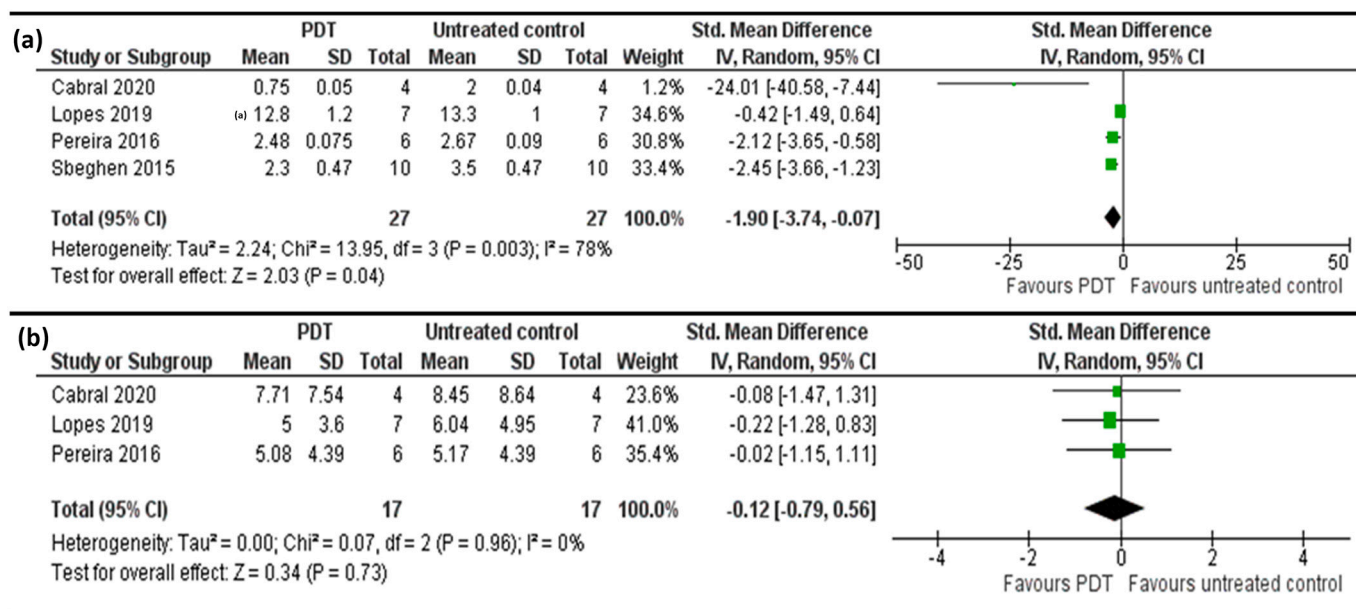

Fig. 3. Forest plots for lesion size (a) and parasite load (b) from PDT studies of preclinical models of CL.

received PDT. On the other hand, three studies were compared regarding the parasite load (in log), totalizing 17 animals per group [39–41]. No statistically significant differences were observed between PDT and untreated control groups (SMD: -0.12, 95% CI: −0.79 to 0.56, *p* = 0.73).

4. Discussion

CL remains a serious public health problem and the development of effective treatment options is urgently required. Hence preclinical studies can provide essential information in respect of the benefits, challenges, and limitations of proposing clinical treatment protocols to manage CL. As CL is part of the group of neglected diseases, fast, safe, and simple treatment options are desirable.

As far as we are concerned, we performed for the first time a systematic review with meta-analysis targeting the use of light-based technologies on CL. The central purpose of this study was to determine whether there is sufficient scientific evidence that this kind of therapy would be able to tackle CL using animal models, which could inspire and guide subsequent clinical studies.

We identified that PDT is the only antimicrobial light-based technology reported to fight CL. Then, we conducted a search in Pubmed looking for "cutaneous leishmaniasis" and "treatment". We noticed that the search for antileishmanial drugs has been growing since 1917. Surprisingly, the first studies about the photodynamic treatment of CL were published in 2003, even though the photodynamic effect was postulated more than 100 years ago [10]. Moreover, the number of these studies is much smaller than those regarding other kinds of

antileishmanial therapies (Fig. 4).

In this review, we noticed that depending on *Leishmania* spp., different animal models were used to induce CL, although most of the studies used females and the promastigote form. Indeed, the immune response to CL depends not only on the host but also on the *Leishmania* species. BALB/c and C57BL/6 are not susceptible to L. *braziliensis*, a species responsible for developing the mucocutaneous form [42,43]. For both animal models, the infection is self-healing as a consequence of L. *braziliensis* to elicit a Th1 primary immune response [42]. Therefore, golden hamsters seem to be the only animals with the potential to be suitable experimental models over this *Leishmania* species [42,44,45].

C57BL/6 mice infected by L. *major* also lead to a self-healing disease because of the predominant Th1 response [46]. The same animal model might develop either a Th1 or Th2 immune response when infected by L. *amazonensis* [42]. On the other hand, BALB/c mice infected by L. *major* or L. *amazonensis* tend to develop chronic diseases due to the susceptibility to infection because of dominant Th2 immune response [42,44,46].

Leishmania parasites, once in the mammalian host, are obligatory intracellular pathogens, multiplying mostly within macrophages [47,48]. For this reason, only a humoral response does not ensure parasite control [49]. Indeed, parasitic load eradication may be a huge challenge using BALB/c mice and L. *major* or L. *amazonensis*, since there is no cellular immune response Th1-mediated [46].

Nevertheless, this type of preclinical study is very important to benefit, in the future, human patients without Th1-mediated immune response, which is strongly associated with the severe CL form [49]. The main purpose of using BALB/c mice for treatment studies relies on the idea that if the therapy has a positive effect on susceptible animals, so it will also be able to have an impact on resistant cases in humans to heal more severe wounds. The natural susceptibility allows the parasite regrowth in case they are not completely eradicated. Thus, any parasite load reduction could be considered promising [44].

The infection site is another key point for the development of CL in preclinical trials. It allows monitoring both the evolution of the lesion and the disease progress. It has been reported that footpad lesions are commonly used for drug screening and/or alternative therapies [44].

Ear infections or CL induced at the base of the tail have been widely employed for vaccine development [44,50]. In this review, half of the studies induced CL in the ear [34–37], followed by three studies infecting the footpad [38,40,41], and one at the base of the tail [39].

Furthermore, PS choice is of great importance. Antileishmanial effect of liposomal chloroaluminum phthalocyanine (AlClPC) was reported against *Leishmania amazonensis* in two studies [39,40]. We found that in both articles, the authors have encapsulated AlClPC into liposomes as a strategy to target the parasite membrane and deliver the PS since it is insoluble on water and aggregation is often an issue.

Other five studies used phenothiazine compounds, such as methylene blue (MB) [38,41] and 3,7-bis(*N,N*-dibutylamino) phenothiazinium bromide (PPA904) [34,35,37]. As cationic agents, both MB and PPA904 can interact better with the negatively charged *Leishmania* membrane. Besides, it seems that if the PS is administered in a cream, a longer incubation time in the dark is necessary to obtain a lower parasitic load [35,37].

On the other hand, ALA (aminolevulinic acid)-PDT has been demonstrated to induce necrosis when L. *major*-infected mice were treated. ALA is converted into protoporphyrin IX (PpIX), an endogenous PS. Although *Leishmania* spp. are unable to produce PpIX from ALA, Akilov et al. suggested that these parasites could take up exogenous PpIX from the host macrophages [36].

Although light doses and irradiances varied slightly among studies, all studies used red light, since PS should be able to absorb the light and transfer energy or charge to molecular oxygen and substrate to produce ROS [10]. However, PpIX could exhibit more pronounced cell killing using blue light due to its more intense absorption at this wavelength [51]. Additionally, six studies performed more than one PDT session [35,37–41]. Thus, we assume that at least two sessions are required to enhance PDT.

The studies were further investigated regarding the risk of bias. Although most of the studies reported baseline characteristics, few or no studies provided information regarding the random sequence generation and allocation concealment. Yet, no studies reported any indication regarding the performance bias and the random outcome assessment. Taking together, it is noteworthy to recommend that future preclinical
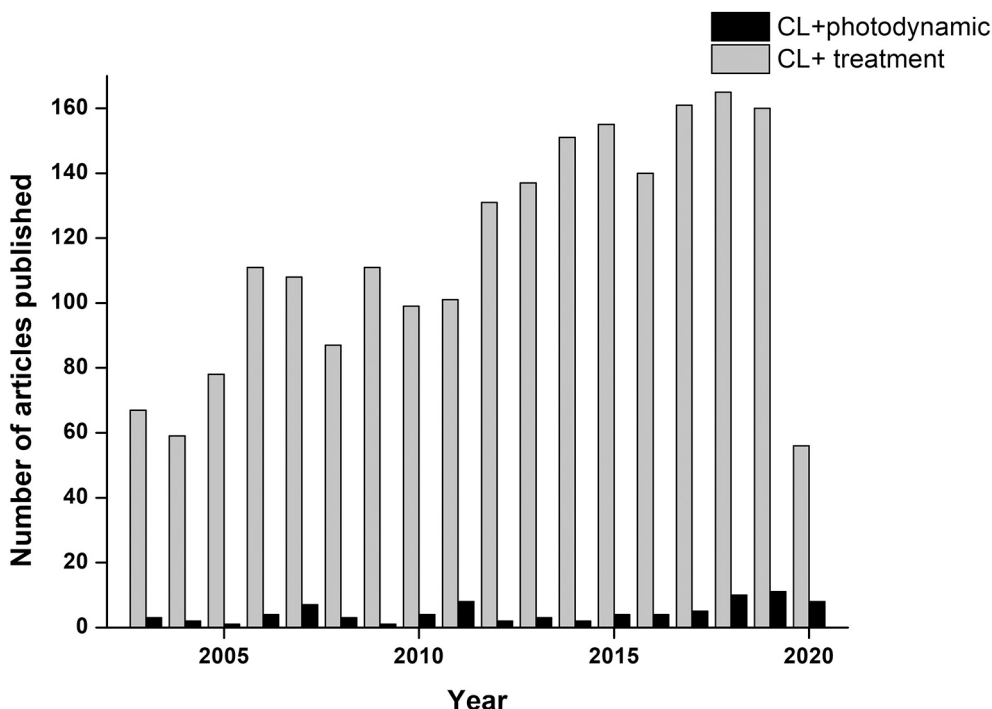

Fig. 4. Number of studies published since 2003 regarding therapies to fight CL.

studies regarding PDT on CL take into account these issues.

In terms of random and blinding housing, although they were not mentioned in the studies, the investigator should be blind. Despite that, we believe that a full-blinded study would be very difficult to accomplish because of the PS color. As a dye, many PSs could stain the skin for days or even weeks depending on the concentration and/or the compound used, making it difficult to blind the investigator. Other domains were mostly assessed as low risk of bias, even though we emphasize the importance of reporting the number of animals evaluated per group in future studies.

To assess if PDT could promote benefits to decrease lesion size and/ or parasite load, we performed the meta-analysis. Although all studies have reported a reduction in the parasite burden, no overall statistically significant differences were observed between untreated control and PDT groups. In contrast, although the methodological heterogeneity has been reflected in high statistical heterogeneity, PDT significantly reduced the lesion size. Therefore, based on the meta-analysis, the main effect of PDT in preclinical trials, regardless of the animal model, PS, light parameters, and the number of PDT sessions would be clinical healing rather than a complete parasitological cure.

These findings suggest not only that the disease is being controlled, but also indicate a possible modulation of the inflammatory response, as reported by Sbeghen et al. [38]. Histopathological analysis of lesions demonstrated a lower number of inflammatory cells on the epidermis. A regenerated dermis with bundles of collagen fibers and reduction of lymphatic capillaries was further observed after PDT. Cabral et al. also suggested an anti-inflammatory effect over pain relief associated with smaller lesions and better clinical presentations [41].

It is noteworthy that in this review we used the untreated group as the comparator since traditional antileishmanial drugs are systemically administered besides promoting adverse effects. Besides, literature is rich in reporting that no pronounced microbial killing is observed when PS or light is used alone in *in vitro* assays. Thus, only three studies reported the parasite load after PS administration in this review [35–37]. As expected, no significant parasite inactivation was observed, regardless of the PS used.

Interestingly, one of the studies showed that the association of oral miltefosine and AlClPC-PDT further decreased the lesion size and the parasite load [40]. This could be an attractive point to be addressed in future studies since PDT has shown synergism with different antimicrobials and could even decrease the recommended dose of the drug [52–54] preventing toxicity to vital organs like the liver, kidneys, *etc.*

Indeed, as previously mentioned the choice of the PS plays an important role in PDT, particularly concerning *in vivo* administration. Successful PDT is highly influenced by PS penetration into the tissue as well as its affinity to accumulate in the target. ROS generation is also strongly dependent on the PS physicochemical properties. Thus, different PSs have been investigated against several medically important pathogens to improve PDT. For example, 1,9-dimethyl-methylene blue (DMMB) is an attractive phenothiazine-based compound because of its capability to produce 21% more singlet oxygen, be more lipophilic, and promote higher microbial inactivation with a lower concentration than MB [55,56]. Besides, the use of longer wavelengths, which promote deeper light penetration into biological tissue, has been a motivation for the development of new PSs. In this regard, tetrapyrrole molecules as bacteriochlorins seem to be promising near-infrared PSs [57]. Further studies are welcome to evaluate the effectiveness of these and other PSs *in vivo* to combat CL.

Even though there is no validated protocol of preclinical PDT towards CL, this systematic review demonstrates that topical PDT reduces the lesion size. This is a very important issue to be addressed as the disfiguring lesions have a huge psychological impact on affected people. Thus, PDT can also improve patients' quality of life and self-esteem, making it a potential ally for the management of CL.

Author Contributions

All authors contributed equally to the manuscript.

Declaration of Competing 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.

Acknowledgments

References

[1] S. Burza, S.L. Croft, M. Boelaert, Leishmaniasis, Lancet 392 (2018) 951–970. Epub 2018/08/17, https://doi.org/10.1016/S0140-6736(18)31204-2.

[2] S.P. Georgiadou, K.P. Makaritsis, G.N. Dalekos, Leishmaniasis revisited: current aspects on epidemiology, diagnosis and treatment, J. Transl. Int. Med. 3 (2015) 43–50, https://doi.org/10.1515/jtim-2015-0002.

[3] World Health Organization, Neglected Tropical Diseases, Available at: https://www.who.int/neglected_diseases/diseases/en/, 2017.

[4] S.L. Croft, P. Olliaro, Leishmaniasis chemotherapy—challenges and opportunities, Clin. Microbiol. Infect. 17 (2011) 1478–1483, https://doi.org/10.1111/j.1469-0691.2011.03630.x.

[5] M. Yanik, M.S. Gurel, Z. Simsek, M. Kati, The psychological impact of cutaneous leishmaniasis, Clin. Exp. Dermatol. 29 (2004) 464–467, https://doi.org/10.1111/j.1365-2230.2004.01605.x.

[6] S.S. Santos, R.V. de Araújo, J. Giarolla, O.E. Seoud, E.I. Ferreira, Searching for drugs for Chagas disease, leishmaniasis and schistosomiasis: a review, Int. J. Antimicrob. Agents 55 (2020) 105906, https://doi.org/10.1016/j.ijantimicag.2020.105906.

[7] A. Ponte-Sucre, F. Gamarro, J.C. Dujardin, M.P. Barrett, R. López-Vélez, R. García-Hernández, A.W. Pountain, R. Mwenechanya, B. Papadopoulou, Drug resistance and treatment failure in leishmaniasis: a 21st century challenge, PLoS Negl. Trop. Dis. 11 (2017), e0006052, https://doi.org/10.1371/journal.pntd.0006052.

[8] C.D. Shaw, J. Lonchamp, T. Downing, H. Imamura, T.M. Freeman, J.A. Cotton, M. Sanders, G. Blackburn, J.C. Dujardin, S. Rijal, B. Khanal, C.J. Illingworth, G. H. Coombs, K.C. Carter, *In vitro* selection of miltefosine resistance in promastigotes of *Leishmania donovani* from Nepal: genomic and metabolomic characterization, Mol. Microbiol. 99 (2016) 1134–1148, https://doi.org/10.1111/mmi.13291.

[9] I. Kevric, M.A. Cappel, J.H. Keeling, New World and Old World *Leishmania* infections: a practical review, Dermatol. Clin. 33 (2015) 579–593, https://doi.org/10.1016/j.det.2015.03.018.

[10] E.M. van der Snoek, D.J. Robinson, J.J. van Hellemond, H.A. Neumann, A review of photodynamic therapy in cutaneous leishmaniasis, J. Eur. Acad. Dermatol. Venereol. 22 (2008) 918–922, https://doi.org/10.1111/j.1468-3083.2008.02805.x.

[11] A.H. Siadat, A. Zolfaghari, Z. Shahmoradi, S. Shariat, K. Sohrabi, Application of laser for treatment of cutaneous leishmaniasis: a review of literature, Lasers Med. Sci. 35 (2020) 1451–1457, https://doi.org/10.1007/s10103-020-03006-1.

[12] C.P. Sabino, M. Wainwright, M.S. Ribeiro, F.P. Sellera, C. Dos Anjos, M.D. S. Baptista, N. Lincopan, Global priority multidrug-resistant pathogens do not resist photodynamic therapy, J. Photochem. Photobiol. B 208 (2020) 111893, https://doi.org/10.1016/j.jphotobiol.2020.111893.

[13] Y. Wang, C.K. Murray, M.R. Hamblin, D.C. Hooper, T. Dai, Antimicrobial blue light inactivation of pathogenic microbes: state of the art, Drug Resist Updat 33–35 (2017) 1–22, https://doi.org/10.1016/j.drup.2017.10.002.

[14] A.J. García-Malinis, A. Milagro Beamonte, L. Torres Sopena, O. García-Callen, P. Puertolas-Villacampa, Y. Gilaberte, Cutaneous sporotrichosis treated with methylene blue-daylight photodynamic therapy, J. Eur. Acad. Dermatol. Venereol. 32 (2018) e90–e101, https://doi.org/10.1111/jdv.14545.

[15] O. Coppellotti, C. Fabris, M. Soncin, M. Magaraggia, M. Camerin, G. Jori, L. Guidolin, Porphyrin photosensitised processes in the prevention and treatment of water- and vector-borne diseases, Curr. Med. Chem. 19 (2012) 808–819, https://doi.org/10.2174/092986712799034905.

[16] C. Zane, E. Facchinetti, M.T. Rossi, C. Specchia, P.G. Calzavara-Pinton, A randomized clinical trial of photodynamic therapy with methyl aminolaevulinate vs. diclofenac 3% plus hyaluronic acid gel for the treatment of multiple actinic keratoses of the face and scalp, Br. J. Dermatol. 170 (2014) 1143–1150, https://doi.org/10.1111/bjd.12844.

[17] R.B.M. de Vries, C.R. Hooijmans, M.W. Langendam, J. van Luijk, M. Leenaars, M. Ritskes-Hoitinga, K.E. Wever, A protocol format for the preparation, registration and publication of systematic reviews of animal intervention studies, Evid. Preclin. Med. 2 (2015) 1–9, https://doi.org/10.1002/ebm2.7.

[18] C.R. Hooijmans, M.M. Rovers, R.B. de Vries, M. Leenaars, M. Ritskes-Hoitinga, M. W. Langendam, SYRCLE's risk of bias tool for animal studies, BMC Med. Res. Methodol. 14 (2014) 43, https://doi.org/10.1186/1471-2288-14-43.

[19] S.M. Viana, F.S. Celes, L. Ramirez, B. Kolli, D.K.P. Ng, K.P. Chang, C.I. de Oliveira, Photodynamic vaccination of BALB/c mice for prophylaxis of cutaneous leishmaniasis caused by *Leishmania amazonensis*, Front. Microbiol. 9 (2018) 165, https://doi.org/10.3389/fmicb.2018.00165.

[20] F. Hoseinipoor, M. Banihashemi, M.R. Jaafari, Zari Javidi, A.A. Azarian, V. M. Goyonlo, The effect of sun radiation on the course of cutaneous leishmaniasis in BALB/c mice, Iran J. Basic Sci. 14 (2010) 145–150 (2010).

[21] O.E. Akilov, M.X. Wu, Y. Jin, Z. Zhou, L.J. Geskin, L.D. Falo, T. Hasan, Vaccination with photodynamic therapy-treated macrophages induces highly suppressive T-regulatory cells, Photodermatol. Photoimmunol. Photomed. 27 (2011) 97–107, https://doi.org/10.1111/j.1600-0781.2011.00578.x.

[22] S. Kumari, M. Samant, P. Khare, P. Misra, S. Dutta, B.K. Kolli, S. Sharma, K. P. Chang, A. Dube, Photodynamic vaccination of hamsters with inducible suicidal mutants of *Leishmania amazonensis* elicits immunity against visceral leishmaniasis, Eur. J. Immunol. 39 (2009) 178–191, https://doi.org/10.1002/eji.200838389.

[23] A.A.L. Sepúlveda, A.M. Arenas Velásquez, I.A. Patiño Linares, L. de Almeida, C. R. Fontana, C. Garcia, M.A.S. Graminha, Efficacy of photodynamic therapy using TiO₂ nanoparticles doped with Zn and hypericin in the treatment of cutaneous Leishmaniasis caused by Leishmania amazonensis, Photodiagn. Photodyn. Ther. 30 (2020) 101676, https://doi.org/10.1016/j.pdpdt.2020.101676.

[24] M.L.F. Silva, P.M. Alves, D.M. Souza, M.V. Silva, J.P. Dos Santos, T.P. Paulino, D. B. Rodrigues, V. Rodrigues Jr., Analysis of macrophage activation markers in an experimental model of cutaneous leishmaniasis treated with photodynamic therapy mediated by 5-aminolevulinic acid, Photobiomodul. Photomed. Laser Surg. 37 (2019) 298–304, https://doi.org/10.1089/photob.2018.4574.

[25] J.B.P. Ribeiro, A.L. Miranda-Vilela, A.A.S. Amorim, R.D. Garcia, J.R. Moreira, C. M. Gomes, G.H.S. Takano, G.M.F. de Oliveira, A.V. Lima, I.C.R. da Silva, R.N. R. Sampaio, Study of the efficacy of N-methyl glucamine antimoniate (SbV) associated with photodynamic therapy using liposomal chloroaluminium phthalocyanine in the treatment of cutaneous leishmaniasis caused by *Leishmania* (*L.*) *amazonensis* in C57BL6 mice, Photodiagnos. Photodyn. Ther. 26 (2019) 261–269, https://doi.org/10.1016/j.pdpdt.2019.04.004.

[26] L.S. Peloi, C.E. Biondo, E. Kimura, M.J. Politi, M.V. Lonardoni, S.M. Aristides, R. C. Dorea, N. Hioka, T.G. Silveira, Photodynamic therapy for American cutaneous leishmaniasis: the efficacy of methylene blue in hamsters experimentally infected with *Leishmania* (*Leishmania*) *amazonensis*, Exp. Parasitol. 128 (2011) 353–356, https://doi.org/10.1016/j.exppara.2011.04.009.

[27] R.A.F. do Nascimento Volpe, T.R. Navascoo, V.N. Dos Reis, N. Hioka, T.C. A. Becker, M.V.C. Lonardoni, S.M.A. Aristides, T.G.V. Silveira, Photodynamic therapy for the treatment of American tegumentary leishmaniasis: evaluation of therapies association in experimentally infected mice with *Leishmania* (*Leishmania*) *amazonensis*, J. Lasers Med. Sci. 9 (2018) 274–282, https://doi.org/10.15171/jlms.2018.49.

[28] D.M. Souza, P.M. Alves, M.L. Silva, T.P. Paulino, H.O. Coraspe, M.M. Mendonça, B. M. Rodrigues, M.V. da Silva, V. Rodrigues Júnior, D.B. Rodrigues, 5-ALA-mediated photodynamic therapy reduces the parasite load in mice infected with *Leishmania braziliensis*, Parasite Immunol. 39 (2017), https://doi.org/10.1111/pim.12403.

[29] P. Escobar, A.M. Vera, L.F. Neira, A.O. Velásquez, H. Carreño, Photodynamic therapy using ultradeformable liposomes loaded with chlorine aluminum phthalocyanine against *L.* (V.) *braziliensis* experimental models, Exp. Parasitol. 194 (2018) 45–52, https://doi.org/10.1016/j.exppara.2018.09.016.

[30] A. Montoya, A. Daza, D. Muñoz, K. Ríos, V. Taylor, D. Cedeño, I.D. Vélez, F. Echeverri, S.M. Robledo, Development of a novel formulation with hypericin to treat cutaneous leishmaniasis based on photodynamic therapy in *in vitro* and *in vivo* studies, Antimicrob. Agents Chemother. 59 (2015) 5804–5813, https://doi.org/10.1128/AAC.00545-15.

[31] O. Akilov, S. Kosaka, T. Hasan, Photochemistry-based immune modulation in the treatment of cutaneous leishmaniasis, Proc. SPIE 7380 (2009) 73803G–1, https://doi.org/10.1117/12.827041.

[32] S. Kosaka, O.E. Akilov, K. O'Riordan, T. Hasan, A mechanistic study of delta-aminolevulinic acid-based photodynamic therapy for cutaneous leishmaniasis, J. Invest. Dermatol. 127 (2007) 1546–1549, https://doi.org/10.1038/sj.jid.5700719.

[33] V.M. Taylor, D.L. Cedeño, D.L. Muñoz, M.A. Jones, T.D. Lash, A.M. Young, M. H. Constantino, N. Esposito, I.D. Vélez, S.M. Robledo, *In vitro* and *in vivo* studies of the utility of dimethyl and diethyl carbaporphyrin ketals in treatment of cutaneous leishmaniasis, Antimicrob. Agents Chemother. 55 (2011) 4755–4764, https://doi.org/10.1128/AAC.00671-11.

[34] E. Latorre-Esteves, O.E. Akilov, P. Rai, S.M. Beverley, T. Hasan, Monitoring the efficacy of antimicrobial photodynamic therapy in a murine model of cutaneous leishmaniasis using *L. major* expressing GFP, J. Biophotonics 3 (2010) 328–335, https://doi.org/10.1002/jbio.201000008.

[35] O.E. Akilov, W. Yousaf, S.X. Lukjan, S. Verma, T. Hasan, Optimization of topical photodynamic therapy with 3,7-bis(di-n-butylamino)phenothiazin-5-ium bromide for cutaneous leishmaniasis, Lasers Surg. Med. 41 (2009) 358–365, https://doi.org/10.1002/lsm.20775.

[36] O.E. Akilov, S. Kosaka, K. O'Riordan, T. Hasan, Parasiticidal effect of delta-aminolevulinic acid-based photodynamic therapy for cutaneous leishmaniasis is indirect and mediated through the killing of the host cells, Exp. Dermatol. 16 (2007) 651–660, https://doi.org/10.1111/j.1600-0625.2007.00578.x.

[37] O.E. Akilov, S. Kosaka, K. O'Riordan, T. Hasan, Photodynamic therapy for cutaneous leishmaniasis: the effectiveness of topical phenothiaziniums in parasite eradication and Th1 immune response stimulation, Photochem. Photobiol. Sci. 6 (2007) 1067–1075, https://doi.org/10.1039/b703521g.

[38] M.R. Sbeghen, E.M. Voltarelli, T.G. Campois, E. Kimura, S.M. Aristides, L. Hernandes, W. Caetano, N. Hioka, M.V. Lonardoni, T.G. Silveira, Topical and intradermal efficacy of photodynamic therapy with methylene blue and light-emitting diode in the treatment of cutaneous leishmaniasis caused by *Leishmania braziliensis*, J. Lasers Med. Sci. 6 (2015) 106–111, https://doi.org/10.15171/jlms.2015.03.

[39] S.C. Lopes, R.A. Silva, M.V. Novais, L.D. Coelho, L.A. Ferreira, P.E. Souza, A. Tedesco, R.B. Azevedo, M.G. Aguiar, M.C. Oliveira, Topical photodynamic therapy with chloroaluminum phthalocyanine liposomes is as effective as systemic pentavalent antimony in the treatment of experimental cutaneous leishmaniasis, Photodiagn. Photodyn. Ther. 28 (2019) 210–215, https://doi.org/10.1016/j.pdpdt.2019.08.020.

[40] J.B.P. Ribeiro, A.L. Miranda-Vilela, D. Graziani, M.R.A. Gomes, A.A.S. Amorim, R. D. Garcia, Filho J. de Souza, A.C. Tedesco, F.L. Primo, J.R. Moreira, A.V. Lima, R.N. R. Sampaio, Evaluation of the efficacy of systemic miltefosine associated with photodynamic therapy with liposomal chloroaluminium phthalocyanine in the treatment of cutaneous leishmaniasis caused by *Leishmania* (*L.*) *amazonensis* in C57BL/6 mice, Photodiagn. Photodyn. Ther. 13 (2016) 282–290, https://doi.org/10.1016/j.pdpdt.2015.08.006.

[41] F.V. Cabral, C.P. Sabino, J.A. Dimmer, I.P. Sauter, M.J. Cortez, M.S. Ribeiro, Preclinical investigation of methylene blue-mediated antimicrobial photodynamic therapy on *Leishmania* parasites using real-time bioluminescence, Photochem. Photobiol. 96 (2020) 604–610, https://doi.org/10.1111/php.13188.

[42] P. Scott, F.O. Novais, Cutaneous leishmaniasis: immune responses in protection and pathogenesis, Nat. Rev. Immunol. 16 (2016) 581–592, https://doi.org/10.1038/nri.2016.72.

[43] G.K. DeKrey, H.C. Lima, R.G. Titus, Analysis of the immune responses of mice to infection with *Leishmania braziliensis*, Infect. Immun. 66 (1998) 827–829, https://doi.org/10.1128/IAI.66.2.827-829.1998.

[44] E.R. Mears, F. Modabber, R. Don, G.E. Johnson, A review: the current *in vivo* models for the discovery and utility of new anti-leishmanial drugs targeting cutaneous leishmaniasis, PLoS Negl. Trop. Dis. 9 (2015), e0003889, https://doi.org/10.1371/journal.pntd.0003889.

[45] R.A. Neal, C. Hale, A comparative study of susceptibility of inbred and outbred mouse strains compared with hamsters to infection with New World cutaneous leishmaniases, Parasitology 87 (1983) 7–13, https://doi.org/10.1017/s0031182000052379.

[46] D. McMahon-Pratt, J. Alexander, Does the *Leishmania major* paradigm of pathogenesis and protection hold for New World cutaneous leishmaniases or the visceral disease? Immunol. Rev. 201 (2004) 206–224, https://doi.org/10.1111/j.0105-2896.2004.00190.x.

[47] J. Alexander, A.R. Satoskar, D.G. Russell, *Leishmania* species: models of intracellular parasitism, J. Cell Sci. 112 (1999) 2993–3002.

[48] J. Alexander, D.G. Russell, The interaction of *Leishmania* species with macrophages, Adv. Parasitol. 31 (1992) 175–254, https://doi.org/10.1016/s0065-308x(08)60022-6.

[49] P. Kaye, P. Scott, Leishmaniasis: complexity at the host-pathogen interface, Nat. Rev. Microbiol. 9 (2011) 604–615, https://doi.org/10.1038/nrmicro2608.

[50] F.L. Ribeiro-Gomes, E.H. Roma, M.B. Carneiro, N.A. Doria, D.L. Sacks, N.C. Peters, Site-dependent recruitment of inflammatory cells determines the effective dose of *Leishmania major*, Infect. Immun. 82 (2014) 2713–2727, https://doi.org/10.1128/IAI.01600-13.

[51] L. Helander, H.E. Krokan, A. Johnsson, O.A. Gederaas, K. Plaetzer, Red versus blue light illumination in hexyl 5-aminolevulinate photodynamic therapy: the influence of light color and irradiance on the treatment outcome *in vitro*, J. Biomed. Opt. 19 (2014), 088002, https://doi.org/10.1117/1.JBO.19.8.088002.

[52] N. Iluz, Y. Maor, N. Keller, Z. Malik, The synergistic effect of PDT and oxacillin on clinical isolates of *Staphylococcus aureus*, Lasers Surg. Med. 50 (2018) 535–551, https://doi.org/10.1002/lsm.22785.

[53] I.T. Kato, R.A. Prates, C.P. Sabino, B.B. Fuchs, G.P. Tegos, E. Mylonakis, M. R. Hamblin, M.S. Ribeiro, Antimicrobial photodynamic inactivation inhibits *Candida albicans* virulence factors and reduces *in vivo* pathogenicity, Antimicrob. Agents Chemother. 57 (2013) 445–451, https://doi.org/10.1128/AAC.01451-12.

[54] J. Chibebe Junior, B.B. Fuchs, C.P. Sabino, J.C. Junqueira, A.O. Jorge, M. S. Ribeiro, M.S. Gilmore, L.B. Rice, G.P. Tegos, M.R. Hamblin, E. Mylonakis, Photodynamic and antibiotic therapy impair the pathogenesis of *Enterococcus faecium* in a whole animal insect model, PLoS One 8 (2013), e55926, https://doi.org/10.1371/journal.pone.0055926.

[55] M. Wainwright, H. Mohr, W.H. Walker, Phenothiazinium derivatives for pathogen inactivation in blood products, J. Photochem. Photobiol. B 86 (2007) 45–58, https://doi.org/10.1016/j.jphotobiol.2006.07.005.

[56] L.M. Pereira, C.M. Mota, L. Baroni, C.M. Bronzon da Costa, J.C.V. Brochi, M. Wainwright, Mineo TWP, G.Ú.L. Braga, A.P. Yatsuda, Inhibitory action of phenothiazinium dyes against Neospora caninum, Sci. Rep. 10 (2020) 7483, https://doi.org/10.1038/s41598-020-64454-x.

[57] B. Pucelik, A. Sułek, J.M. Dąbrowski, Bacteriochlorins and their metal complexes as NIR-absorbing photosensitizers: properties, mechanisms, and applications, Coord. Chem. Rev. 416 (2020) 213340, https://doi.org/10.1016/j.ccr.2020.213340.