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Towards effective natural anthraquinones to mediate antimicrobial photodynamic therapy of cutaneous leishmaniasis

Jesica A. Dimmer [a,b,1,*], Fernanda V. Cabral [c], Susana C. Núñez Montoya [a,b], Martha S. Ribeiro [c]

[a] Universidad Nacional Córdoba. Fac. Cs. Químicas. Dpto. Ciencias Farmacéuticas. Edificio de Ciencias 2, Medina Allende y Haya de La Torre, Ciudad Universitaria. CP, X5000HUA Córdoba, Argentina

[b] Instituto Multidisciplinario de Biología Vegetal (IMBIV), CONICET. Av. Vélez Sarsfield 1666 CP, X5016GCN Córdoba, Argentina

[c] Centro de Lasers e Aplicações, Instituto de Pesquisas Energéticas e Nucleares (IPEN-CNEN), Av. Lineu Prestes 2242, C. Universitária "Armando de Salles Oliveira", CEP 05508-000 São Paulo, SP, Brasil

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ABSTRACT

Background: Cutaneous leishmaniasis (CL) is an important tropical neglected disease with broad geographical dispersion. The lack of effective drugs has raised an urgent need to improve CL treatment, and antimicrobial photodynamic therapy (APDT) has been investigated as a new strategy to face it with positive outcomes. Natural compounds have emerged as promising photosensitizers (PSs), but their use in vivo remains unexplored.

Purpose: In this work, we investigated the potential of three natural anthraquinones (AQs) on CL induced by Leishmania amazonensis in BALB/c mice.

Study Design/Methods: :

Animals were infected and randomly divided into four groups: CG (control, non-treated group), G5ClSor-gL (treated with 5-chlorosoranjidiol and green LED, 520±10 nm), GSor-bL and GBisor-bL (treated with soranjidiol and bisoranjidiol, respectively, exposed to violet-blue LED, 410±10 nm). All AQs were assayed at 10 μM and LEDs delivered a radiant exposure of 45 J/cm^2 with an irradiance of 50 mW/cm^2. We assessed the parasite burden in real time for three consecutive days. Lesion evolution and pain score were assessed over 3 weeks after a single APDT session.

Results: G5ClSor-gL was able to sustain low levels of parasite burden over time. Besides, GSor-bL showed a smaller lesion area than the control group, inhibiting the disease progression.

Conclusion: Taken together, our data demonstrate that monoAQs are promising compounds for pursuing the best protocol for treating CL and helping to face this serious health problem. Studies involving host-pathogen interaction as well as monoAQ-mediated PDT immune response are also encouraged.

Introduction

Cutaneous leishmaniasis (CL) is an infectious, non-contagious disease caused by different species of protozoan parasites of the genus *Leishmania.* It is considered one of the most important neglected tropical diseases with a wide geographical distribution over several countries in the Americas, Africa, Asia, and Europe, resulting in a serious public health problem [1,2].

Following a sandfly bite in exposed areas of skin, a pink papule progresses to a nodule or plaque and eventually forms an ulcer with raised borders. These dermatological conditions lead to severe inflammatory processes, triggering deep ulcerative lesions, compromising healing, and favoring the growth of opportunistic bacteria, resulting in secondary-stage infections [1]. Although CL is not life-threatening, disfiguring lesions and severe disabilities affect the psychological and social aspects of the patient. These complications lead to a highly unproductive population that eventually results in economic loss and the slowdown of a country's development [2,3].

It is well established that parasite species, host immune response, and the clinical manifestation of CL are related to the therapeutic

* Corresponding author at: Universidad Nacional Córdoba. Fac. Cs. Químicas. Dpto. Ciencias Farmacéuticas. Edificio de Ciencias 2, Medina Allende y Haya de La Torre, Ciudad Universitaria. CP, X5000HUA Córdoba, Argentina.

E-mail address: jesica.dimmer@unc.edu.ar (J.A. Dimmer).

[1] Current addres: Centro de Investigaciones y Transferencia Villa María (CIT VM), Instituto Académico Pedagógico de Ciencias Humanas, Universidad Nacional de Villa María, Arturo Jauretche 1555, X5900 Villa María, Córdoba, Argentina. Tel: +549353 – 453 9134.

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response to currently available drugs. In addition, these treatments are limited by high cost, long-term treatment, multiple injections (usually intravenous), and high levels of toxicity and resistance, resulting in low patient compliance with treatment [4,5]. Therefore, the high incidence of treatment failure and the increased number of reported cases lead to an urgent need to further investigate new drugs and alternative therapies to improve CL treatments [1,5].

Our study is focused on the proposal of a local therapy known as antimicrobial photodynamic therapy (APDT), which is based on the association of a photosensitizer (PS) with a light source at a suitable wavelength to generate reactive oxygen species and to kill a wide range of microbial species [6]. Moreover, it is unlikely to cause resistance and, as a local treatment, might be safer and easier to manage compared with systemic administration drugs [7].

Anthraquinones (AQs) are natural photosensitizing drugs extracted from the *Heterophyllaea* genus, native to Argentina and Bolivia [8]. Our previous *in vitro* study demonstrated that the AQs soranjidiol (Sor), bisoranjidiol (Bisor), and 5-chlorosoranjidiol (5ClSor) combined with light-emitting diodes (LEDs) significantly reduced the metabolic activity of *Leishmania amazonensis* promastigotes [9]. These AQs (Fig. 1) have been studied by our group because of their potential as PSs to produce the photoinactivation of bacteria, fungi, and viruses mediated by APDT [10–12]. Their antimicrobial effect could also help to prevent secondary infections produced in CL.

Based on the demand for natural, non-toxic, and effective PSs, our previous studies have motivated us to further investigate the *in vivo* potential of these AQs against CL. We present our preliminary findings on the ability of Sor, Bisor, and 5ClSor to mediate APDT in BALB/c mice infected with *L. amazonensis*, which is one of the most important causative agents of CL and can cause multiple nodular lesions throughout the body, a condition known as diffuse CL [13]. Thus, we assessed the parasite burden in real time for three consecutive days. Lesion evolution and pain score were assessed over 3 weeks after a single APDT session.

Materials and methods

Natural photosensitizers

Three natural AQs were used in this study: Sor, Bisor, and 5ClSor [8]. Purity above 95% was accepted and was checked using HPLC conditions [8]. As the lowest *in vitro* active concentration was 2.5 μM for the three PS [9], we decided to administer 10 μM since biological tissues are more complex structures than cultured cells. Each AQ was prepared in a phosphate-buffered solution (PBS) with less than 1% of dimethyl sulfoxide (DMSO) as a co-solvent. Solutions were sterilized through a 0.22 μm filter membrane.

Parasites

A wild-type strain (MHOM/BR/73/M2269) of *L. amazonensis* was transfected to obtain a recombinant strain expressing the luciferase gene (*La*-LUC) as previously reported [14]. The ATP-bioluminescence assay is based on the reaction between luciferin substrate, molecular oxygen, and adenosine triphosphate (ATP) from viable cells that is catalyzed by the luciferase enzyme (LUC) to produce photons (bioluminescence). Bioluminescence is a non-invasive tool to assess parasite burden in real-time [15,16].

La-LUC promastigotes were grown in 25 cm^2 tissue culture flasks containing M199 medium (Sigma-Aldrich), supplemented with 10% fetal bovine serum (FBS; Gibco™ Invitrogen Corporation), 40 mM HEPES at pH 7.4 (Sigma-Aldrich), hemin (0.005%) (Sigma-Aldrich), penicillin/streptomycin 100 μg/ml (Sigma-Aldrich) and hygromycin at 32 μg/ml. They were incubated at 25 °C for 7 days to obtain stationary growth phase promastigotes.

Animals

Before assays, the protocol was approved by the IPEN Ethical Committee on Animal Use (protocol nº 244/19). Twelve female Balb/C mice (8 weeks old) were infected subcutaneously in the left paw with a suspension of 1×10^6 promastigotes in the stationary growth phase.

APDT

Four weeks after infection and before APDT, mice were randomly divided into four groups (n=3/group): CG (control, non-treated infected group), G5ClSor-gL (infected and treated with 5ClSor and a green LED, 520 ± 10 nm), GSor-bL (infected and treated with Sor), and GBisor-bL (infected and treated with Bisor), the last two were exposed to a violet-blue LED, 410 ± 10 nm. Light groups were not included since only light showed similar inactivation to control group *in vitro* [9].

First, 50 μL of each AQ were administered subcutaneously. The animals were immediately anesthetized for 5 min with 2.5% isoflurane and maintained with 1.5% isoflurane. After 10 min (the pre-irradiation time), lesions were irradiated with a violet-blue or green LED (LEDsaber prototype, Biolambda, Brazil), according to the PS. Both systems were set to deliver a fluence of 45 J/cm^2 (around 50 mW/cm^2, 15 min) on the lesion. The optical power was estimated by using a power meter (LM-01, Coherent, USA). Parameters and wavelengths were selected according to the results of our *in vitro* study. As *in vitro* LD_{90} was approximately 22 J/cm^2, we doubled the light dose to 45 J/cm^2 [9].

Parasite burden

Parasite load was addressed before APDT (2 h) and 24 h, 48 h, and

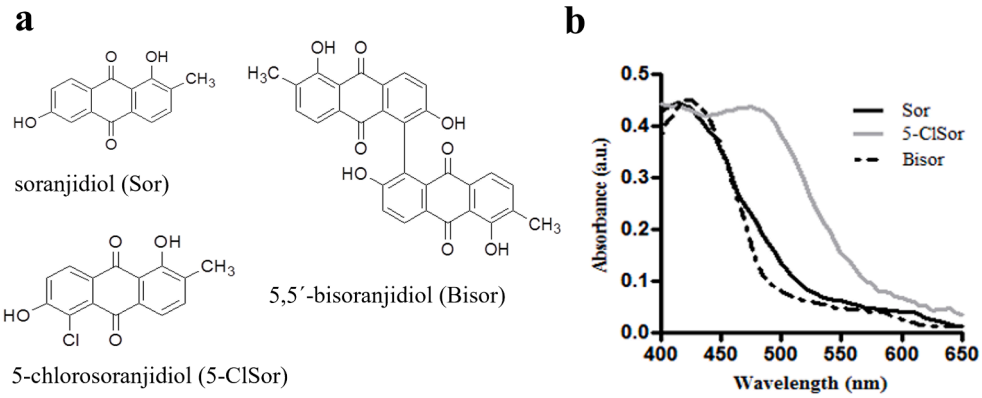

Fig. 1. a) Anthraquinone chemical structures; b) Anthraquinone absorption bands.

72 h post-APDT by bioluminescence imaging using an IVIS Spectrum (Caliper Life Sciences, USA). Firstly, an intraperitoneal injection of 75 mg/kg VivoGlo® Luciferin (Promega Corporation, USA) was administered to mice, followed by the inhalation of 2.5% isoflurane (Cristália, Brazil) to induce anesthesia. The animals were then transferred to the imaging chamber and kept sedated by a 1.5% isoflurane atmosphere. Twenty-min after the luciferin injection, images were acquired. The *La*-LUC photons' emission was quantified within a region of interest (ROI) corresponding to the site of infection in the footpad. The total photon emission of each animal was evaluated with Living Image software version 4.3.1 (Caliper Life Sciences, USA), and results were expressed as radiance (p/sec/cm^2/sr) [17].

Lesion size

Lesion thickness was assessed using a caliper, in which both infected and non-infected paws were measured. Then, lesion size values were addressed as follows in Equation 1:

$$\text{Lesion size}(\%) = \frac{(P_i - P_{ni})}{\overline{P_c}} \quad (1)$$

where P_i is the infected footpad and P_{ni} is the non-infected contralateral one of the same animal and $\overline{P_c}$ is the average of the control group without treatment [17].

Pain evaluation

Pain sensitivity was measured using the Von Frey test. It is a stimulus-evoked method that uses filaments to evaluate the animal's nociceptive sensibility [18]. Filaments vary according to the force scale that corresponds to a pain score, as shown in Table 1.

Statistical analysis

Data on parasite burden and lesion size were compared by a two-way ANOVA of repeated measures, which compares the mean differences among groups that have been split on two independent variables (in our case, treatment, and time). To identify the differences between the two variables, we used the Fisher test as a *post hoc* test. We used uppercase letters to represent statistically significant differences for the first variable (time) and lowercase letters to represent statistically significant differences for the second variable (treatment). For pain evaluation and lesion size, the nonparametric Kruskal-Wallis test was applied, followed by Dunn's post-test. OriginPro software was used for statistical analysis, and data were considered statistically significant when $p < 0.05$.

Results

Due to the immediate effects promoted by APDT, parasite burden was determined 2 h post-treatment and once a day for the following 72 h, so we could properly evaluate the treatment response over time. In Fig 2, we can see that all groups had similar parasite load before and 2 h post-APDT. Twenty-four h following treatment, we noticed a significant increase in parasite burden for CG (around 35%) and GBisor-bL (around 60%), which was sustained until 72 h post-treatment. GSor-bL animals, on the other hand, had a statistically significant higher parasite load 48 h post-APDT. Interestingly, G5Clsor-gL was very effective at maintaining parasite load levels over time. Furthermore, G5ClSor-gL had a statistically significant lower parasite burden (around 30%) than GBisor-bL 72 h post-APDT.

Table 1
Correlation between force (g) and pain score of Von Frey filaments.

Force (g)	Pain Score
>100	1
60–100	2
26–60	3
15–26	4
10–15	5
<10	6

We also assessed treatment considering the clinical manifestations. Fig. 3a displays the evolution of the lesion size during the experimental period. We noticed that in all groups, the lesion size increased over time. Following one week of APDT, GSor-bL showed a significantly smaller lesion size than GBisor-bL. Besides, GSor-bL promoted a significantly reduced lesion size (around 25%) compared to CG and GBisor-bL three weeks post-APDT.

Ulceration on the lesion is also a clinical sign that supports the assessment of lesion progression. We observed that one week after APDT, all the animals from GSor-bL had no ulceration on the lesion, and only one mouse from G5ClSor-gL presented signs of ulceration (Fig. 3b). In contrast, all GBisor-bL animals developed ulcerative lesions similar to CG (Fig 3b). Three weeks after APDT, these ulcers worsened in all groups.

Pain sensitivity was also monitored for 3 weeks. In the first week, although all treated groups showed a smaller pain score than CG, no statistically significant differences were noticed. Indeed, the pain sensitivity remained unchanged for all groups over the experimental period (Fig. 4).

Discussion

This work evaluated the impact of natural AQs on the inactivation of *L. amazonensis in vivo*. Regarding the assessment of antiparasitic PSs, synthetic compounds have produced positive outcomes *in vitro and in vivo* [19–21]. However, the use of natural products as antileishmanial compounds remains unexplored. The benefits of natural treatments include lower toxicity, fewer side effects, and a reduced cost [22]. Moreover, it has been reported that AQs can kill a wide range of microorganisms as well as inhibit different tumor cells [23].

In this study, AQs were tested at low concentrations (10 μM) and were photoactivated at their optimal wavelength with LEDs set to deliver 45 J/cm^2. By comparing the three tested AQs, we observed that monoAQs (Sor and 5ClSor) promoted better results in the control of parasite burden than Bisor. Indeed, three days following APDT, G5ClSor-gL maintained low levels of parasite load as at the beginning of the experimental period, suggesting this compound was very effective in preventing parasite regrowth after treatment. This could be associated with the presence of chlorine in the structure of this AQ, which increases the lipophilicity of the molecule so the PS can be properly internalized by the parasites.

Treatment with Sor-mediated APDT also promoted smaller lesion sizes than CG and GBisor-bL at the end of the experimental period. Yet, no animals in the GSor-bL group showed ulcerations one week-post APDT. These findings indicate an intrinsic effect of Sor in reducing inflammatory processes, even though a parasite regrowth was noticed in this group 3 days after APDT.

In contrast, although Bisor has shown a significantly lower parasite load 48 h following APDT, probably due to a technique artifact, we noticed a worsening of the lesion. This could be explained by the lower uptake of Bisor by the parasites, as we have previously reported [9]. It has been demonstrated that before killing cells, PSs must follow some decisive steps, such as uptake by cells, accumulation in different subcellular organelles, and absorbing light to produce ROS [24,25]. Therefore, despite *in vitro* studies demonstrating that this dimeric AQ produces higher ratios of $O_2^{\cdot-}$ (about six times more than Sor) [26], the generation of ROS is mostly localized outside the cells, thus resulting in a lower antileishmanial activity [24,25]. In addition, due to the lower uptake of this AQ, we hypothesize that skin interaction with ROS could increase inflammation, which, in combination with local

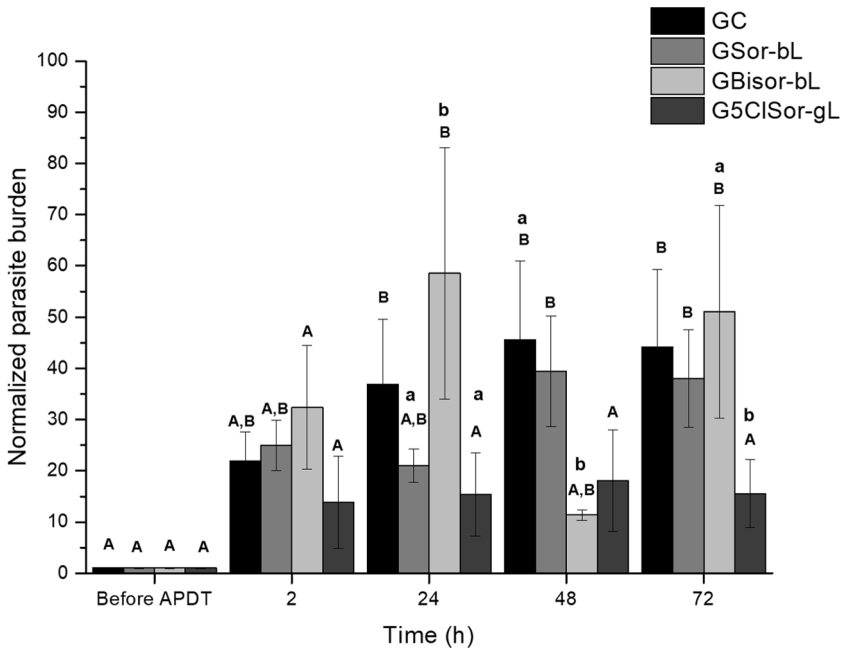

Fig. 2. Mean values ± SEM of the normalized parasite burden. Different uppercase letters (A, B) represent statistically significant differences within-group over time. Different lowercase letters (a, b) denote statistically significant differences among groups within time.

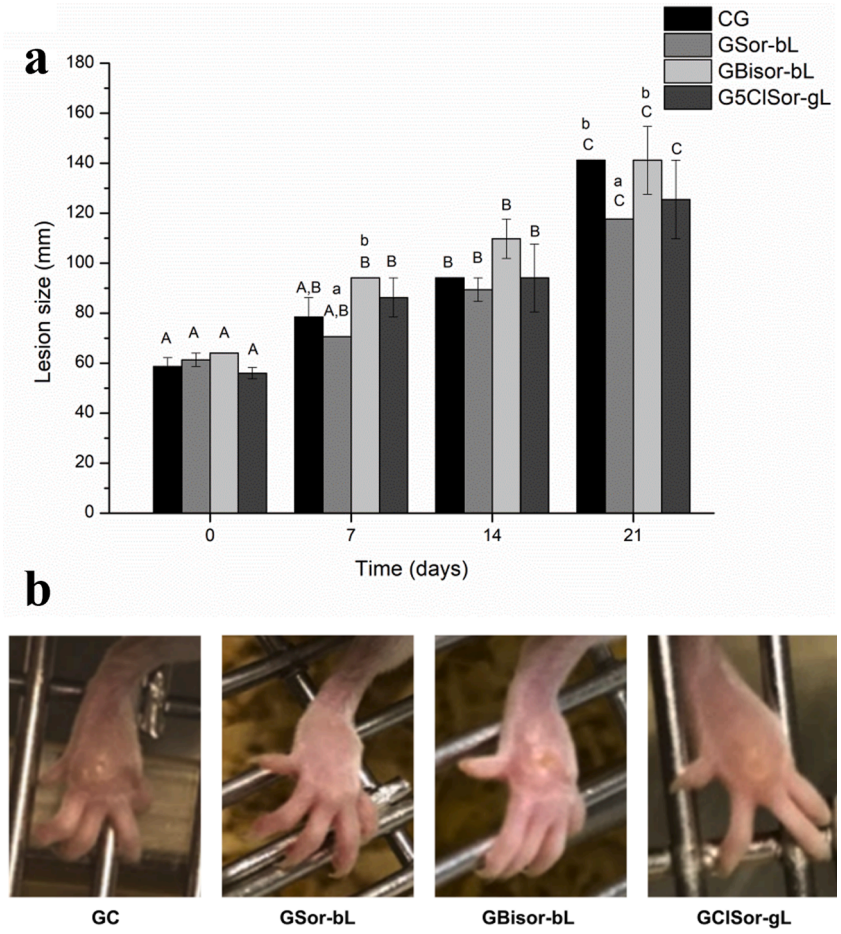

Fig. 3. a) Mean values ± SEM of the lesion size. Different uppercase letters (A, B, C) represent statistically significant differences within-group over time. Different lowercase letters (a, b) denote statistically significant differences among groups within time; b) Clinical aspect of lesions one-week following APDT.

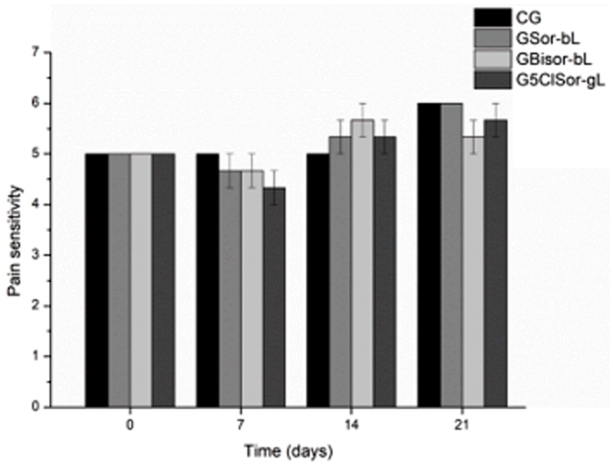

Fig. 4. Mean values ± SEM of the pain sensitivity. No statistically significant differences were detected.

pro-inflammatory cytokines, would produce an increase in edema, hence compromising wound healing [27].

In terms of pain sensitivity, it is well established that infection with *Leishmania* produces hyperalgesia in BALB/c mice. Although pain sensitivity tends to decrease in the first week after APDT (see Fig. 4), AQ-mediated APDT was not able to produce a significant reduction. It was suggested by Borghi and collaborators that the nociceptive response is related to the parasite burden, which induces peripheral production of pro-hyperalgesic mediators [28]. These mediators perpetuate the inflammatory process, contributing to the maintenance of local edema. Thus, we assume that more than one APDT session is necessary to promote pain relief.

It is worth mentioning that BALB/c mice are highly susceptible to infection due to a strong Th2-type immune response, which suggests these animals are unable to control infection themselves [29]. It seems that the control in parasite regrowth promoted by 5ClSor in one single APDT treatment over 3 days (with a very low AQ concentration) is an encouraging result that should be further investigated.

Among the benefits of using monoAQs, it is important to emphasize that they are natural compounds that demonstrate activity at low concentrations and exhibit broad-spectrum antimicrobial activity [10–12]. Compared to other natural PSs, monoAQs are small molecules that have many characteristics of an ideal photosensitizer, including easy eco-friendly, and sustainable synthesis, high photostability, high extinction coefficients, and resistance to photobleaching [30]. Besides, they are more easily dissolved in PBS than curcumin [31], and their large absorption band could match better to LEDs' emission than hypericin [32]. Indeed, LEDs are promising light sources for PDT, as they present some advantages over lamps and lasers. The emission spectrum of a lamp is very broad, but antimicrobial PDT requires wavelengths resonant with the absorbance of the PS. On the other hand, lasers emit coherent narrow beams that present a risk to the eye and often illuminate a very small area.

The difference between both monoAQs, besides the presence of chlorine, was the wavelength used to photoactivate these compounds. The LED was chosen according to the AQ absorbance (see Fig. 1). Although green light penetrates deeper than violet-blue light into biological tissue, the wavelength should not be an issue for PDT of topical infections [32]. Indeed, the photodynamic response leading to microbial killing happens whenever the PS absorbance matches the light emission. Our results demonstrated that Sor-mediated PDT was able to reduce the lesion size compared to the control, whereas 5ClSor maintained low levels of parasite load at the first 72 h post-treatment. Taken together, these exciting results encourage the combination of both Sor and 5ClSor with proper LEDs since synergism between other AQs such as Rubiadin and Sor was previously demonstrated [33].

It is important to highlight that the violet-blue wavelength has an intrinsically specific antimicrobial effect due to Leishmania's endogenous chromophores, even though Silva and collaborators showed that animals from the light-only group did not result in a significant reduction in parasite burden [34]. Indeed, it is well known that only violet-blue light can inactivate different pathogens. However, the exposure time increases substantially compared to antimicrobial photodynamic therapy mediated by exogenous photosensitizers [35]. Indeed, Zhang and collaborators reduced the fungal load in infected mouse burns by 1.75-logs following 80 min of irradiation [36]. Therefore, our results correspond to an inherent effect of AQs' photodynamic action.

A limitation of this study was the murine model used to induce cutaneous leishmaniasis by *L. amazonensis*. Although BALB/c mice are the most widely used, this model is extremely susceptible to this *Leishmania* species. As aforementioned, these animals are unable to control infection if left untreated, simulating an immunosuppressed individual. In addition, *Leishmania* parasites can adapt to different environments, evade the host immune system, become resistant to drugs, and proliferate very fast [1,5,32]. For these reasons, it is very difficult to completely eradicate the parasite load. Here, we proposed to monitor parasite load in the first 72 h. We intended to identify whether we would find a pattern of parasite behavior facing one AQ-PDT application depending on the tested AQ. The results obtained suggest that new experiments should include repeated applications and long-term monitoring. Future directions could also combine monoAQ-mediated PDT with conventional systemic antileishmanials in lower doses.

In conclusion, our data demonstrate that monoAQs are promising compounds for pursuing the best protocol for treating CL and helping to face this serious health problem. Studies involving host-pathogen interaction as well as monoAQ-mediated PDT immune response are also encouraged.

CRediT authorship contribution statement

Jesica A. Dimmer: Conceptualization, Formal analysis, Investigation, Methodology, Visualization, Writing – original draft. **Fernanda V. Cabral:** Investigation, Methodology, Visualization, Writing – review & editing. **Susana C. Núñez Montoya:** Funding acquisition, Resources, Writing – review & editing. **Martha S. Ribeiro:** Conceptualization, Formal analysis, Funding acquisition, Project administration, Resources, Supervision, Validation, Visualization, Writing – review & editing.

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References

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

[2] World Health Organization (WHO) (2022). Global Health Observatory (GHO) data. Avaliable at: https://www.who.int/data/gho/data/themes/topics/gho-ntd-leishmaniasis. Accessed on: 25 October 2022.

[3] F. Bailey, K. Mondragon-Shem, L.R. Haines, A. Olabi, A. Alorfi, J.A. Ruiz-Postigo, J. Alvar, P. Hotez, E.R. Adams, I.D. Vélez, W. Al-Salem, J. Eaton, Á. Acosta-Serrano, D.H. Molyneux, Cutaneous leishmaniasis and co-morbid major depressive disorder: a systematic review with burden estimates, PLoS Negl. Trop. Dis. 13 (2019) 1–22. https://www.doi.org/10.1371/journal.pntd.0007092.

[4] K. Seifert, Structures, targets and recent approaches in anti-leishmanial drug discovery and development, Open Med. Chem. J. 5 (2011) 31–39. https://www.doi.org/10.2174/1874104501105010031.
[5] D. Caridha, B. Vesely, K. van Bocxlaer, B. Arana, C.E. Mowbray, S. Rafati, S. Uliana, R. Reguera, M. Kreishman-Deitrick, R. Sciotti, P. Buffet, S.L. Croft, Route map for the discovery and pre-clinical development of new drugs and treatments for cutaneous leishmaniasis, Int. J. Parasitol. Drugs Drug Resist. 11 (2019) 106–117. https://www.doi.org/10.1016/j.ijpddr.2019.06.003.
[6] F. Cieplik, D. Deng, W. Crielaard, W. Buchalla, E. Hellwig, A. Al-Ahmad, T. Maisch, Antimicrobial photodynamic therapy–what we know and what we don't, Crit. Rev. Microbiol. 44 (2018) 571–589. https://www.doi.org/10.1080/1040841X.2018.1467876.
[7] M.R. Hamblin, Antimicrobial photodynamic inactivation: a bright new technique to kill resistant microbes, Curr. Opin. Microbiol. 33 (2016) 67–73. https://www.doi.org/10.1016/j.mib.2016.06.008.
[8] J.A. Dimmer, S.C. Núñez-Montoya, C.S. Mendoza, J.L. Cabrera, Photosensitizing anthraquinones from *Heterophyllaea lycioides* (Rubiaceae), Phytochemistry 137 (2017), 94–1, https://www.doi.org/10.1016/j.phytochem.2017.02.003.
[9] J. Dimmer, F.V. Cabral, C.P. Sabino, C.R. Silva, S.C. Núñez-Montoya, J.L. Cabrera, M.S. Ribeiro, Natural anthraquinones as novel photosentizers for antiparasitic photodynamic inactivation, Phytomedicine 61 (2019), e152894. https://www.doi.org/10.1016/j.phymed.2019.152894.
[10] L.R. Comini, S.C. Núñez Montoya, P.L. Pérez, G.A. Arguello, I. Albesa, J.L. Cabrera, Antibacterial activity of anthraquinone derivatives from *Heterophyllaea pustulata* (Rubiaceae), J. Photochem. Photobiol. B 102 (2011) 108–114. https://www.doi.org/10.1016/j.jphotobiol.2010.09.009.
[11] B.S. Konigheim, L.R. Comini, S. Grasso, J.J. Aguilar, J. Marioni, M.S. Contigiani, S. C. Núñez Montoya, Determination of non-toxic and subtoxic concentrations of potential antiviral natural anthraquinones, Lat. Am. J. Pharm. 31 (2012) 51–56.
[12] J. Marioni, M.A. da Silva, J.L. Cabrera, S.C. Núñez-Montoya, M.G. Paraje, The anthraquinones rubiadin and its 1-methyl ether isolated from *Heterophyllaea pustulata* reduces *Candida tropicalis* biofilms formation, Phytomedicine 23 (12) (2016) 1321–1328. https://www.doi.org/10.1016/j.phymed.2016.07.008.
[13] S.M. Christensen, A.T. Belew, N.M. El-Sayed, W.L. Tafuri, F.T. Silveira, D. Mosser, Host and parasite responses in human diffuse cutaneous leishmaniasis caused by *L. amazonensis*, PLOS Negl. Trop. Dis. 13 (3) (2019), e0007152. https://www.doi.org/10.1371/journal.pntd.0007152.
[14] J.Q. Reimao, C.T. Trinconi, J.K. Yokoyama-Yasunaka, D.C. Miguel, S.P. Kalil, S. R. Uliana, Parasite burden in *Leishmania (Leishmania) amazonensis*-infected mice: validation of luciferase as a quantitative tool, J. Microbiol. Methods 93 (2013) 95–101. https://www.doi.org/10.1016/j.mimet.2013.02.007.
[15] L. Paloque, N. Vidal, M. Casanova, A. Dumètre, P. Verhaeghe, D. Parzy, N. Azas, A new, rapid and sensitive bioluminescence assay for drug screening on *Leishmania*, J. Microbiol. Methods 95 (2013) 320–323. https://www.doi.org/10.1016/j.mimet.2013.09.006.
[16] 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) 1–23. https://www.doi.org/10.1371/journal.pntd.0003889.
[17] 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 (2019) 604–610. https://www.doi.org/10.1111/php.13188.
[18] J.R. Deuis, L.S. Dvorakova, I. Vetter, Methods used to evaluate pain behaviors in rodents, Front. Mol. Neurosci. 10 (2017) 1–17. https://www.doi.org/10.3389/fnmol.2017.00284.
[19] 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://www.doi.org/10.1016/j.exppara.2011.04.009.
[20] 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 (Sb V) associated with photodynamic therapy using liposomal chloroaluminium phthalocyanine in the treatment of cutaneous leishmaniasis caused by *Leishmania (L.) amazonensis* in C57BL6 mice, Photodiagnosis Photodyn. Ther. 26 (2019) 261–269. https://www.doi.org/10.1016/j.pdpdt.2019.04.004.
[21] 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 TiO_2 nanoparticles doped with Zn and hypericin in the treatment of cutaneous Leishmaniasis caused by *Leishmania amazonensis*, Photodiagnosis Photodyn. Ther. 30 (2020), 101676. https://www.doi.org/10.1016/j.pdpdt.2020.101676.
[22] T.P. Kubrak, P. Kołodziej, J. Sawicki, A. Mazur, K. Koziorowska, D. Aebisher, Some natural photosensitizers and their medicinal properties for use in photodynamic therapy, Molecules 27 (2022) 1192. https://www.doi.org/10.3390/molecules27041192.
[23] M. Kasipandi, B. George, T. Parimelazhagan, H. Abrahamse, Role of photoactive phytocompounds in photodynamic therapy of cancer, Molecules 25 (2020) 4102. https://www.doi.org/10.3390/molecules25184102.
[24] I.O.L. Bacellar, T.M. Tsubone, C. Pavani, M.S. Baptista, Photodynamic efficiency: from molecular photochemistry to cell death, Int. J. Mol. Sci. 16 (2015) 20523–20559. https://www.doi.org/10.3390/ijms160920523.
[25] L. Benov, Photodynamic therapy: current status and future directions, Med. Princ. Pract. 24 (2015) 14–28. https://www.doi.org/10.1159/000362416.
[26] L.R. Comini, S.C. Núñez Montoya, M. Sarmiento, J.L. Cabrera, G.A. Argüello, Characterizing some photophysical, photochemical and photobiological properties of photosensitizing anthraquinones, J. Photochem. Photobiol. A Chem. 188 (2007) 185–191. https://www.doi.org/10.1016/j.jphotochem.2006.12.011.
[27] L.R. Castellano, D.C. Filho, L. Argiro, H. Dessein, A. Prata, A. Dessein, V. Rodrigues, Th1/Th2 immune responses are associated with active cutaneous leishmaniasis and clinical cure is associated with strong interferon-gamma production, Hum. Immunol. 70 (2009), 383-90, https://www.doi.org/10.1016/j.humimm.2009.01.007.
[28] S.M. Borghi, V. Fattori, I. Conchon-Costa, P. Pinge-Filho, W.R. Pavanelli, W. A. Verri, Leishmania infection: painful or painless? Parasitol. Res. 116 (2017) 465–475. https://www.doi.org/10.1007/s00436-016-5340-7.
[29] J. Barbi, F. Brombacher, A.R. Satoskar, T cells from Leishmania major-susceptible BALB/c mice have a defect in efficiently up-regulating CXCR3 upon activation, J. Immunol. 181 (2008) 4613–4620. https://www.doi.org/10.4049/jimmunol.181.7.4613.
[30] J Dimmer, Fotosensibilizadores Fotodinámicos Naturales Y Potenciales Aplicaciones Terapéuticas, Universidad Nacional de Córdoba., In Spanish, 2017. Doctoral thesis, http://hdl.handle.net/11086/15282.
[31] B. Zheng, D.J. McClements, Formulation of more efficacious curcumin delivery systems using colloid science: enhanced solubility, stability, and bioavailability, Molecules 25 (2020) 2791, https://doi.org/10.3390/molecules25122791.
[32] M. Varzandeh, R. Mohammadinejad, K. Esmaeilzadeh-Salestani, A. Dehshahri, A. Zarrabi, A. Aghaei-Afshar, Photodynamic therapy for leishmaniasis: Recent advances and future trends, Photodiagnosis Photodyn. Ther. 36 (2021), 102609, https://doi.org/10.1016/j.pdpdt.2021.102609.
[33] N.B. Vittar, L.R. Comini, I.M. Fernadez, E. Agostini, S.C. Núñez-Montoya, J. L. Cabrera, V.A. Rivarola, Photochemotherapy using natural anthraquinones: rubiadin and Soranjidiol sensitize human cancer cell to die by apoptosis, Photodiagnosis Photodyn. Ther. 11 (2014) 182–192. https://www.doi.org/10.1016/j.pdpdt.2014.02.002.
[34] 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.
[35] C.P. Sabino, M. Wainwright, C. dos Anjos, F.P. Sellera, M.S. Baptista, N. Lincopan, M.S. Ribeiro, Inactivation kinetics and lethal dose analysis of antimicrobial blue light and photodynamic therapy, Photodiagnosis Photodyn. Ther. 28 (2019) 186–191, https://doi.org/10.1016/j.pdpdt.2019.08.022.
[36] Y. Zhang, Y. Zhu, J. Chen, Y. Wang, M.E. Sherwood, C.K. Murray, M.S. Vrahas, D. C. Hooper, M.R. Hamblin, T Dai, Antimicrobial blue light inactivation of Candida albicans: in vitro and in vivo studies, Virulence 7 (2016) 536–545, https://doi.org/10.1080/21505594.2016.1155015.