ARTÍCULO ORIGINAL

Cell accumulation and antileishmanial effect of exogenous and endogenous protoporphyrin IX after photodynamic treatment

Jairo E. Mateus[1], Wilfredo Valdivieso[1], Indira P. Hernández[1],
Fernando Martínez[2], Edgar Páez[2], Patricia Escobar[1]

[1] Centro de Investigación de Enfermedades Tropicales (CINTROP), Escuela de Medicina, Departamento de Ciencias Básicas, Universidad Industrial de Santander, Bucaramanga, Colombia
[2] Centro de Investigación en Catálisis (CICAT), Escuela de Química, Universidad Industrial de Santander, Bucaramanga, Colombia

Introduction: Photodynamic therapy (PDT) using 5-aminolevulinic acid-induced protoporphyrin IX (ALA-PpIX) constitutes an interesting alternative for cutaneous leishmaniasis treatment.

Objective: To evaluate the production of PpIXbased on the administration of ALA and MAL and the effect of ALA-PDTat cellular level on non-infected and infected THP-1 cells using *Leishmania* (*Viannia*) *panamensis* or *Leishmania* (*Leishmania*) *infantum* (syn *Leishmania chagasi*) parasites.

Materials and methods: Protoporphyrin IX (PpIX) production and mitochondrial colocalization were evaluated by confocal microscopy. Cell toxicities were evaluated after treatment with the compounds, followed by light irradiation (597-752 nm) at 2.5 J/cm^2 fluency using a colorimetric MTT assay for THP-1 cells and a standard microscopic analysis of parasites. Results were expressed as compound concentration activity against 50% of cells or parasites (CC_{50} or IC_{50}).

Results: ALA or MAL induced an endogenous PpIX with a red fluorescence localized mainly in the mitochondria inside human cells. ALA and MAL-PDT induced a similar range of toxicities on THP-1 cells (CC_{50} 0.16±0.01mM and 0.33±0.019 mM, respectively) without any apparent inhibition of intracellular parasites in the infected cells as compared to untreated controls. Exogenous PpIX-PDT was toxic to THP-1 cells (CC_{50} 0.00032±0.00002 mM), *L. (L.) infantum* (IC_{50} 0.003±0.0001 mM) and *L. (V.) panamensis* (IC_{50} 0.024±0.0001 mM) promastigotes.

Conclusions: Despite the effectiveness of exogenous PpIX on promastigotes and the production of PpIX by human infected cells, treatment with ALA or MAL before irradiation was unable to completely destroy *L. (L.) infantum* or *L. (V.) panamensis* intracellular amastigotes.

Key words: Aminolevulinic acid, photochemotherapy, *Leishmania (Viannia) panamensis*, *Leishmania infantum*.

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Acumulación celular y efecto anti-*Leishmania* de la protoporfirina IX exógena y endógena después del tratamiento fotodinámico

Introducción. El tratamiento fotodinámico con ácido 5-aminolevulínico como inductor de la protoporfirina IX (ALA-PpIX) constituye una alternativa interesante en el tratamiento de la leishmaniasis cutánea.

Objetivo. Evaluar la producción de protoporfirina IX (PpIX) a partir de la administración de ALA o MAL y el efecto de la PDT con ALA a nivel celular en células THP-1 no infectadas e infectadas con *Leishmania* (*Viannia*) *panamensis* o *Leishmania* (*Leishmania*) *infantum* (syn. *Leishmania chagasi*).

Materiales y métodos. La producción de protoporfirina IX y su 'colocalización' mitocondrial se evaluaron mediante microscopía 'confocal'. Se evaluó la toxicidad celular después del tratamiento con los compuestos y la aplicación de irradiación de luz (597-752 nm) en una fluencia de 2,5 J/cm^2 mediante el empleo de la prueba colorimétrica con metil-tiazol-tetrazolio (MTT) en las células, y de métodos microscópicos estándar en los parásitos. Los resultados se expresaron como la concentración del compuesto activo en el 50 % de las células o parásitos (CC_{50} o CI_{50}).

Resultados. El ácido aminolevulínico o el metil-5-aminolevulinato indujeron la protoporfirina IX endógena en células humanas, y se observó fluorescencia de color rojo en las mitocondrias. La actividad

del ácido aminolevulínico y del metil-5-aminolevulinato utilizados con terapia fotodinámica fue similar en las células THP-1 (CC_{50} 0,16±0,01 mM y 0,33±0,019 mM, respectivamente) y, aparentemente, no inhibió los parásitos en las células infectadas, en comparación con los controles. El tratamiento exógeno con protoporfirina IX y terapia fotodinámica fue tóxico para las células THP-1 (CC_{50} 0,00032 ±0,00002 mM) y para los promastigotes de *L. (L.) infantum* (IC_{50} 0,003±0,0001 mM) y *L. (V.) panamensis* (CI_{50} 0,024±0,0001 mM).

Conclusiones. A pesar de la 'fotoactividad' del tratamiento con protoporfirina IX en promastigotes y de su producción después del tratamiento con ácido aminolevulínico y metil-5-aminolevulinato en las células infectadas con *Leishmania*, no se observó daño en los amastigotes presentes en las células de *L. (L.) infantum* o *L. (V.) panamensis*.

Palabras clave: ácido aminolevulínico, fotoquimioterapia, *Leishmania* (*Viannia*) *panamensis*, *Leishmania infantum*.

Leishmaniasis is a heterogeneous parasitic disease with a wide spectrum of clinical forms such as the cutaneous (CL), the muco-cutaneous (MCL) and the visceral (VL) forms. It is a vector borne disease caused by several species of the genus *Leishmania*, a digenetic parasite that proliferates as motile promastigotes in the gut of sandflies and as amastigotes inside vertebrate-host macrophages. Leishmaniasis is considered a serious public health problem worldwide, as it is endemic in tropical and subtropical regions in more than 88 countries. Almost 12 million people are currently infected, with an annual reported incidence of 1-1.5 million cases of CL and 0.5 million cases of VL (1,2). Penta-valent antimonials (Sb^{V}, sodium stibogluconate, meglumine antimoniate) followed by amphotericin B (AmB), pentamidine isothionate, paromomycin and miltefosine constitute the major drug options for the disease. These drugs present significant drawbacks such as toxicity and side effects, high costs, low availability, variable effectiveness and high risk of resistance (3,4). The use of topical treatments using ointments or cream formulation of paromomycin, or physical therapies (electrotherapy, thermotherapy, cryotherapy and photodynamic therapy or PDT) constitute interesting alternative options which must be studied carefully, especially in the case of New World leishmaniasis (5,6).

The use of PDT is now widely accepted in derma-tology against different types of skin conditions produced by cancer, aged-related skin degeneration or pathogens (7-9). PDT is based on the use of a photosensitizer agent which absorbs light energy after interaction with visible light and in presence of molecular oxygen generates reactive oxygen species (ROS) like singlet oxygen and the consequent cell damage or cell death (7-9). In leishmaniasis, various PS have been used so far for PDT, and some of them, such as porphyrins, phenothiaziniums analogues, carbaporphyrinketals, phthalocyanines (Pc) methylene blue and endog-enous 5-aminolevulinic acid-induced protoporphyrin IX (ALA-PpIX), have demonstrated antileishmanial activity in clinical and experimental models (10-16).

ALA-PDT constitutes one of the most successful prodrugs used in cancer (17-20). Different topical formulations of ALA, methyl-5-aminolevulinate (MAL) or other ALA-derivatives have been extensively used for treatment of superficial lesions such as skin malignancies, actinic keratosis, acne vulgaris, psoriasis or skin infections caused by bacteria or fungi (17-20). MAL is the active component of Metvix®, a topical cream formulation containing the hydrochloride salt of methyl-5-aminolevulinate, a lipophilic methyl ester of ALA, with photosensitizer (PS) prodrug activity (21,22).

ALA is an endogenous metabolite, which is naturally synthesized in the mitochondria from glycine and succinyl-coenzyme. After several enzymatic reactions, it leads to the formation of the heme group through the incorporation of Fe^{++} into PpIX by the action of a ferrochelatase enzyme. Since the rate of ALA formation is regulated by the amount of the concentration of the produced heme (by down-regulation of transcription and translocation of the enzyme ALA synthase), when ALA is administrated externally, in the case of ALA-PDT (at 570-670 nm), the feedback regulation is bypassed, the capac-ity of the ferrochelatase enzyme to attach iron

Corresponding author:
Patricia Escobar, Centro de Investigación de Enfermedades Tropicales (CINTROP), Escuela de Medicina, Departamento de Ciencias Básicas, Universidad Industrial de Santander, Km 2 vía al Refugio, Sede UIS Guatiguará, Piedecuesta, Colombia
Telephone-Fax: (577) 634 4000, extension 3565
pescobarwww@yahoo.co.uk

decreases and a higher concentration of PpIX is accumulated inside the cells becoming a powerful photosensitizer for PDT (23).

Recent clinical outcomes of PDT using ALA-PDT in patients with CL have been promising, especially on those produced by Old World *Leishmania* species, which are refractory to conventional treatments (12-14). In addition, promising results of ALA-PDT were obtained in BALB/c mice infected with *L. major* (11). The animals were treated topically with one dose of 20% ALA and PDT. Although there was not a complete parasite inhibition, a significant reduction of the parasite loads (24.5 folds compared with the ALA non-irradiated group), a decrease in the percentage of macrophages (from 31.4% to 2.7%), and necrosis and tissue destruction were observed 7 days after ALA-PDT (11). Nonetheless, the clinical outcome was not related with *in vitro* results, since no intracellular amastigote elimination was observed.

In order to evaluate the effect of ALA-PDT at the cell level using New World *Leishmania* species, the production of PpIX based on the administration of ALA and MAL by infected and non-infected THP-1 cells using *Leishmania* (*Viannia*) *panamensis* or *Leishmania* (*Leishmania*) *infantum* (syn. *Leishmania chagasi*) parasites was tested. Since no quantitative analysis was carried out to measure the levels of ALA or MAL induced-PpIX (endogenous), positive control experiments with exogenous PpIX were performed.

Materials and methods

Reagents and compounds

ALA hydrochloride (ALA), methyl 5-aminolevulinate hydrochloride (MAL synonym 5-aminolevulinic acid methyl ester), PpIX, phorbol 12-mirystate 13-acetate (PMA), AmB, 3-(4,5-dimethylthiazolyl)-2,5-diphenyltetrazolium bromide (MTT) and haemin were purchased from Sigma-Aldrich (St. Louis, USA). Dimethyl sulphoxide (DMSO) was obtained from Carlo ErbaReagenti (Rodano, Italy). RPMI 1640 culture medium and fetal calf serum (FCS) were obtained from Gibco (Grand Island, NY, USA). LysoTracker Green DND-26 was obtained from Molecular Probes, Eugene, OR, USA. The mitochondrial probe JC-1 (5,5', 6,6'-tetrachloro-1,1',3,3'-tetraethyl benzimidazolyl carbocyanineiodide) was kindly donated by Dr. Susana Fiorentino from the *Grupo de Inmunobiología y Biología Celular*, Pontificia Universidad Javeriana, Bogotá, Colombia. Stock solutions of ALA and MAL were prepared in RPMI 1640 medium without fetal calf serum. PpIX was dissolved in DMSO. Work solutions were prepared with RPMI 1640 medium immediately before the experiments. The final concentration of the DMSO in work solution was less than 0.5%.

Cells and parasites

Human acute monocytic leukemia THP-1 cells (ATCC TIB 202) were maintained in RPMI 1640 culture medium supplemented with 10% heat inactivated (hiFCS) at 37°C in 5% CO_2 humidified atmosphere. Cells were transformed to the adherent phenotype by treatment with PMA (10 ng/mL) for 72 h. *L. chagasi* (MHOM/BR/74/PP75) currently classified as *L. (L.) infantum* (24) and *L. (V) panamensis* (MHOM/PA/71/LS94) promastigotes were maintained by serial passage in RPMI 1640 supplemented with 10% hiFCS (v/v) and haemin (25 mg/L) at 28°C. For cell infections, transformed THP-1 cells in 16-well Lab-Tek™ chamber slides (Life Technologies, Paisley, UK) were incubated with stationary-phase promastigotes at a ratio of 1:10 for 24 h at 37°C for *L. (L.) infantum* or 33°C for *L. (V.) panamensis*. The percentage of infected cells was variable in each experiment from 50-70% in *L. (V.) panamensis* and from 60-90% for in *L. (L.) infantum*. The numbers of amastigotes per cell were not determined in this study.

Fluorescence microscopy analysis

Infected and non-infected THP-1 cells were incubated with three fold dilutions of ALA and MAL (0.16- 5.0 mM) or exogenous PpIX (0.0062- 0.1 mM). Previous experiments using ALA, MAL and PpIX showed that lower concentrations did not induce fluorescence and higher concentrations than those used in this study were toxic in the dark for THP-1 cells. After 5 h, cells were washed twice with PBS (pH 7.2) to remove the excess of compounds and they were analyzed microscopically using an Olympus FV1000 confocal microscope. The sample was excited with a laser at a 488 nm wavelength (λ) and the emission was separated with a dichroic mirror at λ 580 nm. The images were displayed with a red pseudocolor.

For intracellular localization, cells were incubated with 1.6 mM ALA or MAL. After 5 h, cells were washed twice and incubated with 10 µg/mL JC-1 or 100 nMLysoTracker® Green DND-26 for 10 min. For the mitochondrial analysis, confocal images were taken using a red (PpIX) and green (JC-1) pseudocolor.

For the lysosomal analysis, cells were examined using an epifluorescence microscope (Nikon Eclipse E400) with UV-2A filter (Ex=330-380, DM=400, BA=420) and B-2A filter (Ex=450-490, DM=500, BA=515).

Images were photographed using a charge-coupled device (CCD) color digital camera Nikon Coolpix 5000. Green (organelle) and red (PS) fluorescence images were merged using Adobe PhotoShop CS4 software. Colocalization appears as yellow-orange color. For exogenous PpIX internalization, promastigotes and infected and non-infected cells were incubated with PpIX (0.0062- 0.1-mM) for 24 h. The cell internalization was evaluated by epifluorescence microscopy as described above.

PDT assays on parasites

Late exponential growth promastigotes were incubated in the dark with ALA and MAL (0.16- 5.0 mM), exogenous PpIX (0.0003-0.3 mM) and AmB (0.03-1.0 µM) at 28°C. After 24 h, the parasites were washed once with culture medium after centrifugation at 3,000 rpm for 20 min and re-suspended with normal medium. The cells were irradiated at a light fluency of 2.5 J/cm^2 using a photo reactor equipped with four lamps (50W, 120V) and filters (Edmund Industrial Optics) to select a spectral range from 597 to 752 nm. Control cells were maintained without irradiation. Inhibition of parasite growth was microscopically determined 24 h post-irradiation by counting parasite numbers in a haemocytometer and calculated by comparison to untreated controls. For intracellular amastigotes, infected THP-1 cells were incubated with ALA and MAL (0.002-5 mM), exogenous PpIX (0.006-1.66 mM) and AmB (0.03-1.0 µM) for 48 h. Control cells were maintained untreated. The cells were irradiated as described above. The percentage of infected and non-infected cells was microscopically counted on methanol fixed and Giemsa-stained slides, 72 h post-irradiation. The parasite inhibition was determined by comparison to untreated controls. The concentration able to inhibit 50 (IC$_{50}$) percent of promastigotes or infected cells was calculated by regression analysis (MSxlfit software; ID Business Solutions, Guildford, UK). AmB was used as antileishmanial control drug active with and without light irradiation.

PDT assay on mammalian host cells

Transformed THP-1 cells were incubated with three fold dilutions of ALA or MAL (0.01-45.0 mM), exogenous PpIX (0.000001-0.015 mM) for 24 h at 37°C in 5% CO_2 humidified atmosphere. The cells were irradiated as described above and cell viability was evaluated by a MTT reduction assay which measures the mitochondrial dehydrogenase activity of surviving cells. A solution of 5 mg/mL MTT was added for 4 h and formazan crystals were dissolved with DMSO. Optical densities (OD) were measured in a microplate reader (Sensident Scan Merck) at a wavelength of 580 nm. The percentage of THP-1 cell toxicity was calculated: Cytotoxicity (%) = 1-(optical density treatment group/optical densitycontrol group) x 100. The cytotoxic concentration for 50% (CC$_{50}$) of cells was calculated by sigmoidal regression analysis. THP-1 cells were incubated with serial dilution of AmB (3.3-90 µM) as stated above and were used as a control for the presence of a photoinactive compound.

Statistical analysis

The statistical significance was determined from at least two experiments using the two-tailed unpaired Student's t-test. A p value <0.05 was considered as statistically significant.

Results

ALA and MAL treatment induced a red fluorescence by infected and non-infected THP-1 cells.

An emission of red fluorescence in the cell cytoplasm, but not in the nucleus, with a diffuse pattern was observed in both *Leishmania*-infected and non-infected THP-1 cells (figure 1a and b). No qualitative differences between ALA and MAL red fluorescence were observed (data not shown). The cellular PpIX production with ALA or MAL (from 0.16 mM) reached a maximum at 1.6 mM. No red fluorescence was observed on untreated cells by *L. (L.) infantum* or *L. (V.) panamensis* promastigotes. However, parasites were able to internalize exogenous PpIX as is shown in figure 1c.

ALA and MAL-induced PpIX was localized mainly in mitochondria.

A red fluorescence after ALA and MAL treatment was overlaid with the green mitochondrial probe in *Leishmania*-infected and non-infected THP-1 cells (figures 1d-f). A low lysosome colocalization was also observed (figure 1g). The mitochondrial localization of PpIX after ALA treatment has been also demonstrated extensively in cancer cells and in J774.2 macrophages infected with *Leishmania major* as described above (11).
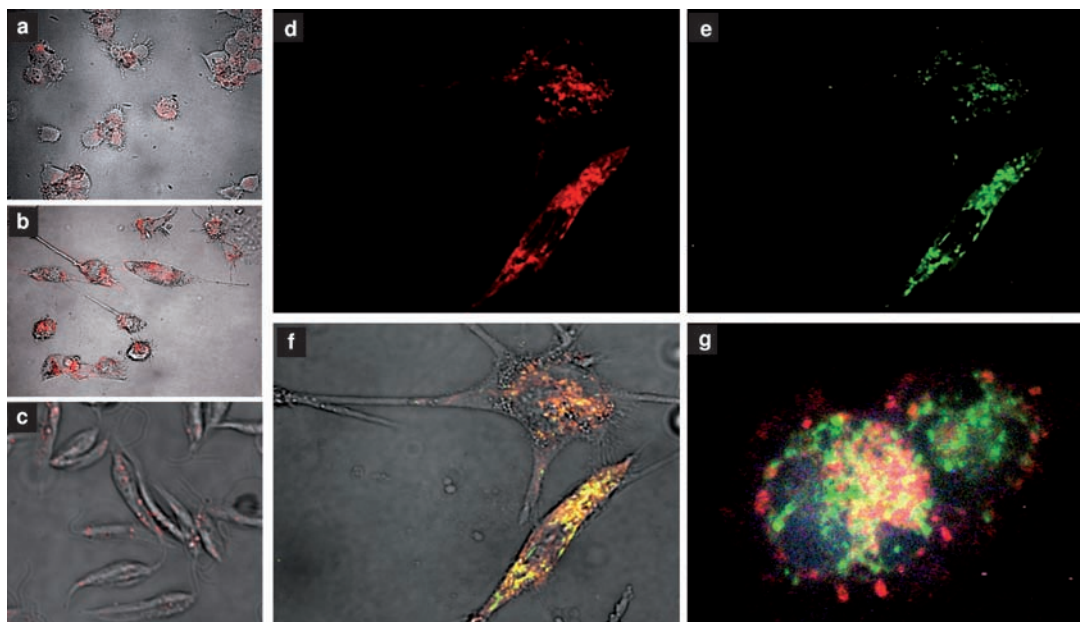
Figure 1. PpIX generation and localization in response to ALA. Microphotographs showed red fluorescence after 1.6 mM ALA for 5 h in a) non-infected and b) *Leishmania*-infected THP-1 cells; c) internalization of exogenous PpIX 0.1 mM by *L.* (*L.*) *infamtum* promastigotes; (d, e, f) colocalization of endogenous PpIX and JC-1 Green mitochondrial probe on *Leishmania*-infected THP-1 cells; d) PpIX red fluorescence; e) JC-1 staining; f) overlay of red and green channels showing colocalization of the two colors; g) green and red fluorescence indicating low colocalization of endogenous PpIX with LysoTracker® Green DND-2. These pictures are representative of at least 10 fields observed in two independent experiments.

ALA, MAL and exogenous PpIX induced photodamage on non-infected THP-1 cells after PDT.

Cell photodamage was induced after ALA and MAL-PDT (CC_{50} 0.16±0.01 mM and CC_{50} 0.33±0.019 mM, respectively). Some toxicity in non-irradiated cells at the maximum concentration evaluated was observed (figure 2). Exogenous PpIX-PDT was toxic to THP-1 cells (CC_{50} of 0.00032±0.00002 mM) and low toxicities (around 20%) were observed in non-irradiated cells (figure 2).

No photo damage was observed after ALA and MAL-PDT on promastigotes.

As expected, *L. (L.) infantum* and *L. (V.) panamensis* promastigotes were not sensitive to ALA and MAL-PDT. Probably, they were unable to metabolize ALA and MAL to produce PpIX. In contrast, exogenous PpIX-PDT was phototoxic to *L. (L.) infantum* (IC_{50} 0.003±0.0001 mM and IC_{90} 0.004±0.0004 mM) and *L. (V.) panamensis* (IC_{50} 0.024±0 .0001 mM and IC_{90} 0.014±0.002 mM) promastigotes (figure 3).

Photodamage in infected host cells was observed after ALA and MAL-PDT.

As described above, the incubation of THP-1 (infected and non-infected) with ALA and MAL produces PpIX in observable quantities. PpIX produced from ALA and MAL in infected THP-1 cells did not produce enough phototoxic effect on intracellular amastigotes of *L. (L.) infantum* and *L. (V.) panamensis* to decrease the percentage of infection, i.e., there was no parasite elimination. In contrast, the numbers of THP-1 cells infected after treatment was greater than in untreated controls.

### No differences were observed between ALA and MAL-PDT activities (P>0.05).

There were no differences in the toxicities displayed by ALA or MAL compounds.

Antileishmanial activity of amphotericin B reference drug

Amphotericin B (AmB) was active against promastigotes and intracellular amastigotes of *L. (V.) panamensis* and *L. (L.) infantum* without significant differences with or without PDT. The antiparasitic activities of AmB in PDT untreated (-) versus PDT treated (+) parasites are shown in figure 3.

Discussion

Photodynamic therapy (PDT) is based on the ability of certain compounds (or photosensitizers) to react to light and produce radical oxygen species
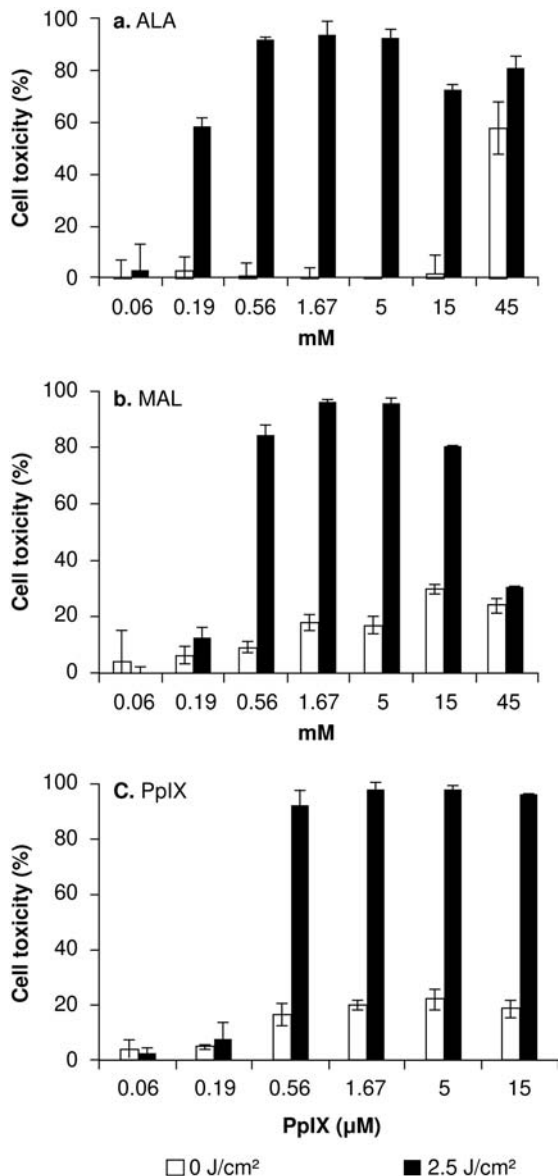
Figure 2. ALA, MAL and PpIX-PDT cell toxicities. PMA-transformed THP-1 cells were incubated for 24 h with increasing concentration of compounds and irradiated at 2.5 J/cm^2. Control cells were kept in the dark (0 J/cm^2). After 24 h, the percentage of cell toxicity was calculated by comparison with untreated controls. The results were expressed as the mean ± SD from two experiments (N=8).

which kill the target cell (7-9). ALA and MAL are two of the most used endogenous photosensitizers and are precursors of PpIX, a high photosensitive product (23). As it is known, parasites of the genus *Leishmania* lack several of the enzymes involved in the PpIX synthesis and treatment with ALA or MAL will not produce a phototoxic effect after PDT (24). Accordingly, in this study, no intra-parasite red fluorescence or phototoxicity were observed when

promastigotes were incubated with ALA or MAL before irradiation, but in contrast, when incubated with exogenous PpIX, red fluorescence and phototoxicity were observed. *L. (L.) infantum* promastigotes were more photosensitive to exogenous PpIX than *L. (V.) panamensis* promastigotes, demonstrating once again the interspecies intrinsic variation in drug sensitivity showed by some antileishmanial drugs, such as miltefosine, to which *L. donovani* (the main species involved in Old World leishmaniasis) was more sensitive than other *Leishmania* species. *Leishmania* parasites have been photosensitive to different exogenous photosensitizers like Al and Zn phthalocyanines, carboporphyrins, and benzophenoxazine (10,16,25,26).

The strategy for the use of endogenous ALA or MAL against intracellular parasites (it is known that amastigotes cannot produce PpIX either) (24), is to evaluate the dynamic of PpIX production by the infected host cells after ALA treatment because if it is produced by the cell, it could be internalized by the parasite. Production of PpIX was observed in both infected and non-infected differentiated THP-1 cells. In a previous report, using a *Leishmania* model, Akilov, *et al.* (11), demonstrated ALA-PpIX production in *L. major*-infected or non-infected J774.2 cells (immortalized murine BALB/c macrophage cells). In those experiments, a low diffuse pattern associated with cell membrane and mitochondria in non-infected cells and low level diffuse fluorescence with bright spots of amastigotes in infected ones were observed. Interestingly, intracellular amastigotes, but not extracellular ones, were able to accumulate time-increased concentration of PpIX (11). Production of PpIX based on ALA or MAL has been described in several cell lines (27,28).

Cellular localization is an important issue, since it is related with the mechanism of cell death trigger. Photosensitizers localized within mitochondria are mostly associated with the apoptotic (some autophagic) death mechanism involved, i.e., caspase (or caspase-independent) activation and mitochondrial dysfunction (potential disruption of mitochondrial membrane and cytochrome C release) giving rise to morphological changes: bleeding, chromatin condensation and alteration of cell adhesion molecules (29-34). Confocal images show overlapping of PpIX red fluorescence and MitoTracker Green fluorescence which strongly suggest PpIX mitochondrial localization in infected and non-infected THP-1 cells. On the other hand, low lysosomal localization was observed.

a. *L. panamensis*

b. *L. infantum*

☐ 0 J/cm² ■ 2.5 J/cm²
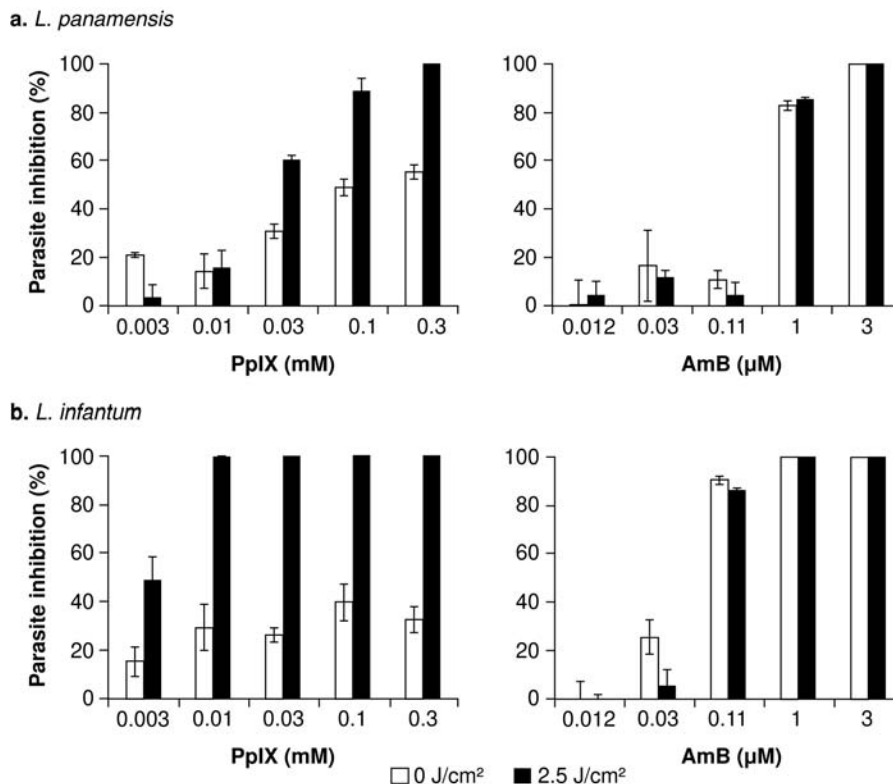
Figure 3. Exogenous PpIX-PDT antiparasitic-activities. Promastigotes of a) *L. (V.) panamensis,* and b) *L. (L.) infantum* were incubated for 48 h with increasing concentration of the compounds and irradiated at 2.5 J/cm². Control parasites were kept in the dark (0 J/cm²). After 24 h, the percentage of parasite inhibition was calculated by comparison with untreated controls. Amphotericin B (AmB) was used as non-phototoxic and reference antileishmanial drug. The results were expressed as the mean ± SD from two experiments (N=8).

This sub-cellular localization is consistent with phototoxicity assays' results. All compounds evaluated (ALA, MAL and PpIX) triggered photo-toxicity in the non-infected host cell, in agreement with previous works (27,28). In this study, the amount of PpIX produced by the cells after ALA or MAL treatment was not quantified, so it was not possible to do a correlation between the activities (at CC_{50} level) achieved with these compounds and those achieved with exogenous PpIX on the cells, i.e., it cannot be said that using 0.16 mM ALA correlates with 0.00032 mM PpIX concentrations occurring inside the cells.

As described above, infected cells also produce PpIX based on ALA or MAL, with a low lysosomal and high mitochondrial localization. When irradiated, PpIX produced from ALA and MAL in infected THP-1 cells did not produce enough phototoxic effect on intracellular amastigotes of *L. (L.) infantum* and *L. (V.) panamensis* to decrease the percentage of infection, i.e., there was no parasite elimination. In contrast, the numbers of THP-1 cells infected after treatment were greater than on the untreated controls. Other studies showed PpIX generation from ALA using other tumor macrophage lines and determined that the amount of PpIX internalized by the intracellular parasites is not sufficient to cause photodamage (11). Interestingly, it has been reported that ALA derivatives with alkyl chains are more easily internalized and more phototoxic than ALA itself (30), but no differences between ALA and MAL were observed. A poorly enhanced internalization (due to just one carbon elongation in the lateral chain) or differences in the selectivity of the esterase enzymes could be involved in the lack of ALA vs. MAL differences shown in this study.

As a conclusion, treatment with ALA or MAL *in vitro* before irradiation with visible light was unable to completely destroy *L. (L.) infantum* or *L. (V.) panamensis* intracellular amastigotes infecting THP-1 cells. A fluorescent photosensitizer at the mitochondrial level was observed in *Leishmania*-infected and non-infected cells. Exogenous PpIX-PDT was toxic to THP-1 cells and free forms of

parasites, but elimination of intracellular amastigotes was difficult to define with the methodology used in this study. PDT by ALA and MAL-induced porphyrin in the host cells could mediate the death of intracellular parasites probably due to host cell destruction. The response of *L. (L.) infantum* and *L. (V.) panamensis* to ALA-PDT showed some similarities with the response induced by ALA-PDT on *L. major* parasites (16). However, differences in the active doses have to be taken into account in case of a formulation for topical treatment of New Word *Leishmania* species.

Conflict of interest

None declared.

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