Electronic Supplementary Information

Photodynamic effect of zinc porphyrin on promastigote and amastigote forms of *Leishmania braziliensis*

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1. Effect of ZnP on Promastigotes Growth in the Dark

ZnTE-2-PyP^{4+} (ZnP) was synthesized by alkylation of the precursor Zn(II) *meso*-tetrakis(2-pyridyl)porphyrin and purified as previously reported by Viana et al. [1] ZnP concentration in all stock solutions was spectrophotometrically determined using the molar absorptivity of the Soret band (ε_{425nm} = 288,403 L/mol·cm). [2] Promastigote forms (1 × 10^6 cells/mL) were incubated in 24-well plates in the dark at 26 °C in Schneider's medium supplemented with 10% FBS in the absence or presence of different concentrations of ZnP (3.1 – 50 μM). Cell density was evaluated daily using a Neubauer counting chamber. The ZnP concentration that inhibited cell growth by 50% (IC_{50}) was estimated after 72 h of culture by linear regression analysis using

SPSS 18.0 software (SPSS Inc., Chicago, USA). The tests were performed in triplicate in two independent assays.

ZnP in the absence of light was able to inhibit the growth of promastigote forms of *L. braziliensis* in a concentration-dependent way (Figure ESI 1). After 72 h of treatment, for the highest concentration tested (50 μM) it was observed an inhibitory effect on parasite density reaching about 60%. Lower concentrations such as 12.5 μM and 25 μM had little effect on cell growth, with inhibition percentage of up to 15%. The calculated IC_{50} for ZnP in the dark was 45 μM.

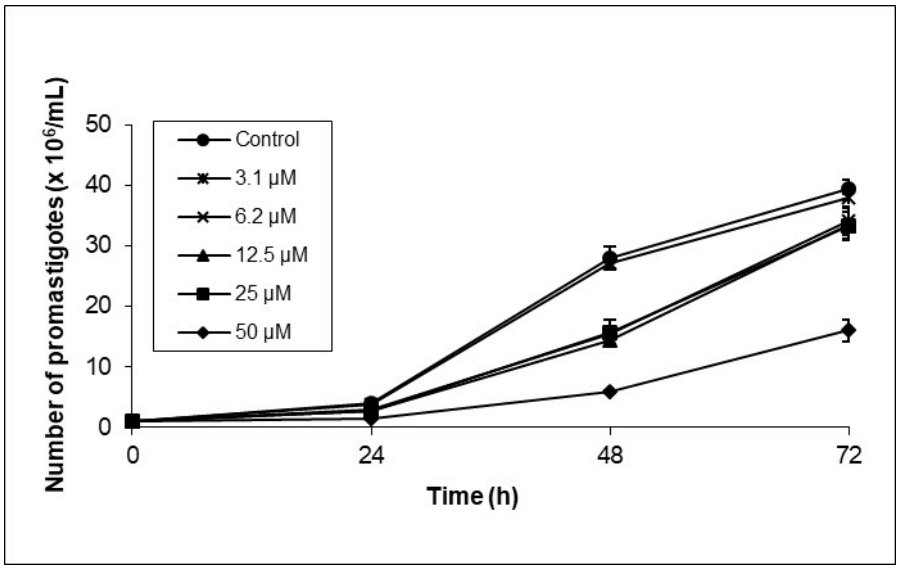

Figure ESI 1 – Multiplication of promastigotes forms of *L. braziliensis* incubated with ZnP in the absence of light.

2. Recovery Assay

The capability of *L. braziliensis* promastigotes to recover after photodynamic treatment with ZnP was also analyzed. For this, after incubation with ZnP photosensitizer and application of light irradiation for 5 min, cells were transferred to the fresh culture medium, and the cell growth was assessed after 24 h of treatment. To calculate the growth percentage, the number of control cells was taken as 100% of cell growth. As shown in Figure ESI 2, the cells submitted to the previous treatment with ZnP and irradiated were unable to resume growth in both concentrations of ZnP used. On the other hand, the light irradiated group in absence of ZnP was able to recover cell growth at similar rates of control cells.

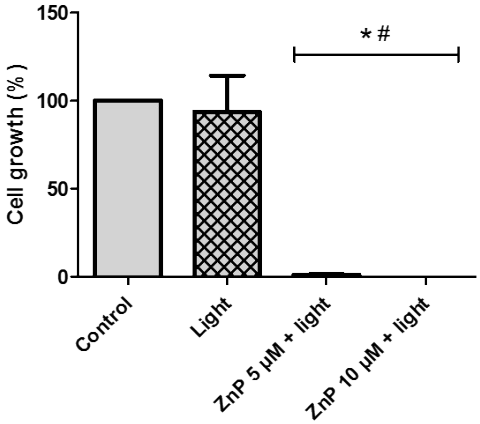

Figure ESI 2 – Cell growth of *L. braziliensis* promastigotes evaluated trough cell counting performed 24 h after treatment. Notes: Groups statistically significant *(p < 0.05)* when compared to the control (*), and light treatment (#).

3. Effect on Parasite Mitochondrial Membrane Potential

Rh 123 was applied to verify alterations in the mitochondrial membrane potential of photodynamically treated parasites (Figure ESI - 3). The red line is a visual guide to follow the increase/decrease of the median of fluorescence compared to control. The higher the median of fluorescence for treated parasites, the more positive the variation index is, indicating that a hyperpolarization has occurred.

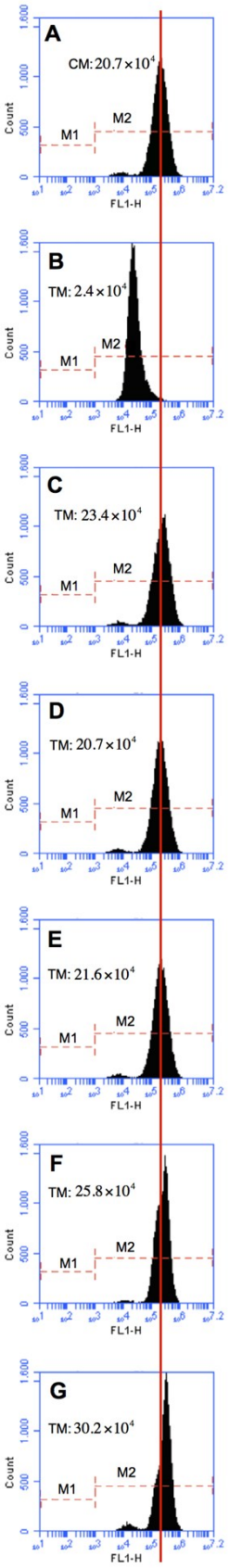

Figure ESI 3 - Fluorescence histograms of *L. braziliensis* promastigotes after ZnP + light treatment indicating labeling with rhodamine 123. M1 – unlabeled cells; M2 – labeled cells. Notes: **A** – Untreated cells: negative control; **B** – cells treated with H_2O_2 (positive control); **C** –

Treatment with light; **D** – Treatment with 5 μM ZnP in the dark; **E** – Treatment with 10 μM ZnP in the dark; **F** – Treatment with 5 μM ZnP + light; **G** – Treatment with 10 μM ZnP + light. TM – median of fluorescence for treated parasites; CM – median of fluorescence for control group.

References

1. O. Viana, M. Ribeiro, A. Rodas, J. Rebouças, A. Fontes and B. Santos, Comparative Study on the Efficiency of the Photodynamic Inactivation of Candida albicans Using CdTe Quantum Dots, Zn(II) Porphyrin and Their Conjugates as Photosensitizers, *Molecules*, 2015, **20**, 8893–8912.

2. L. Benov, I. Batinic-Haberle, I. Spasojevic and I. Fridovich, Isomeric N-alkylpyridylporphyrins and their Zn(II) complexes: Inactive as SOD mimics but powerful photosensitizers, *Arch. Biochem. Biophys.*, 2002, **402**, 159–165.