

Cell death of *Leishmania amazonensis* promastigotes induced by photodynamic therapy



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Leishmania amazonensis is an etiological agent of American cutaneous or mucocutaneous leishmaniasis. Photodynamic therapy (PDT) is a current approach to treat leishmaniasis. In this study we investigated mechanisms of cell death in *L. amazonensis* promastigotes after PDT by flow cytometry. Infective-stage of *L. amazonensis* metacyclic promastigotes was isolated from stationary cultures in 96 well plates, and 107 parasites were incubated with MB (50 μ M and 100 μ M) during 10 min. The samples were irradiated using a 630 nm-LED ($P=100$ mW and 260 mW) during 5 min. Following PDT, the promastigotes of the parasites were incubated with annexin V conjugated to fluorescein isothiocyanate (FITC) and propidium iodide (PI) for 15 min according to the Annexin V-FITC labeling for kit apoptosis detection (eBioscience). The reading of 5000 events independent was standard for all tests. Our results showed a similar fluorescence percentage of parasites stained when PDT was performed with 50 μ M MB (85.8%) or 100 μ M MB (82.7%) and irradiated with $P=100$ mW. For LED power of 260 mW, the fluorescence of parasites stained with Annexin V and PI was 79.4% and 72.1% (50 μ M and 100 μ M MB, respectively). These findings indicate that PDT using MB induces apoptosis.

<http://dx.doi.org/10.1016/j.pdpdt.2015.07.050>

Total biomass of multispecies-biofilm after Photodynamic Therapy mediated by nanoemulsion-phthalocyanine



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Oral candidosis is the most common fungal infection caused by biofilm of *Candida* spp., especially *C. albicans*, but bacterial species contribute to the formation of this biofilm. This study evaluated the total biomass of multispecies-biofilm of *Streptococcus mutans*, *Candida albicans* and *Candida glabrata* submitted to Photodynamic Therapy (PDT) mediated by aluminum-chloride-phthalocyanine encapsulated in cationic nanoemulsion (FC-Cl-Al-NE) associated with 660nm-LED light. Standard suspensions (106–107 CFU/mL) of these microorganisms were transferred to wells of microtiter plates for the multispecies-biofilm formation (48 h at 37 °C in candle jars). The biofilm was treated for 30 minutes with FC-Cl-Al-NE at 31.8 mM. The biofilm was irradiated with a light dose of 39.3 J/cm² (10.7 mW, 21.84 mW/cm², 30 min). Additional biofilms were treated only with FC-Cl-Al-NE or light, and controls consisted of untreated biofilms. Then, each sample was evaluated by measuring the total biomass using the crystal violet staining assay (absorbance at 570 nm). Data were analyzed by ANOVA and Tukey

($\alpha=0.05$). No significant difference ($p=0.340$) was verified among groups, demonstrating that PDT was not able to reduce the total biomass of the biofilm. With the parameters employed, PDT was not able to reduce the total biomass of the biofilm evaluated.

<http://dx.doi.org/10.1016/j.pdpdt.2015.07.051>

A novel regimen for perioral dermatitis by photodynamic therapy



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Background: A chronic condition with unpredictable flare-ups, perioral dermatitis (POD) occurs mostly in women aged 16–45 years. Some patients are not relieved by the acknowledged medications including antibiotics, keratolytic, anti-inflammatory and protective barrier cream. These patients confront pain, psychological bother and social/occupational limitations. Being efficient for a wide variety of infectious and inflammatory diseases photodynamic therapy (PDT) holds a good chance to cure POD.

Materials and methods: This is a 3 case series of women of 22–48 and 50 years old who had POD during 1 and 3 years that was not cured by medications. They asked for PDT in order to try and cease their suffering.

Results: Up to 5 years following PDT the patients discontinued medications, redness decreased and eruption disappeared.

Conclusion: In these 3 patients a single PDT treatment cleared POD. This data is encouraging but not sufficient. Further study is warranted.

<http://dx.doi.org/10.1016/j.pdpdt.2015.07.052>

Resistance to PDT by oral cancer cells: Analysis of autophagy and apoptosis inhibition



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Oral cancer (OC) is a malignant tumor with high morbidity and mortality. High frequency of OC cases develops resistance to the standard treatments. Photodynamic therapy (PDT) has been used in OC with good outcomes; however there is no information about the process of resistance to PDT. One of the tumor resistance pathways is the inhibition of apoptosis and autophagy through bcl-2 and mTOR expression, respectively. The aim of this study was to verify whether OC cells that survival after ALA-mediated PDT express bcl-2 and mTOR markers. After 24 h of ALA-mediated PDT (4 h ALA incubation, irradiation with LED, 5.86 J/cm², 10 J, 150 mW, 150 s), cells viability measured by MTS was 4.8%; 94% of these cells were positive for TUNEL. After 5 days culture, survival cells exhibited growth rate lower than controls ($p<0.01$), but mean viability was 71.1%. Analysis using double immunofluorescence for TUNEL and mTOR revealed that the survival PDT-treated cells (TUNEL–) had frequency of mTOR expression (78.4%) higher than non-treated PDT cells (9.2%), ALA-treated (8.0%) and LED-treated cells (19.2%) ($p<0.01$). None of PDT-treated cells (TUNEL–/bcl2+) expressed bcl-2. We concluded that survival PDT-treated OC cells maintain its proliferation capacity after the treatment, and may develop resistance to autophagy through mTOR pathway.

<http://dx.doi.org/10.1016/j.pdpdt.2015.07.053>