I.03 - LIPIDOMICS ANALYSIS OF LEISHMANIA AMAZONENSIS FOLLOWING PHOTOXIDATIVE STRESS

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INTRODUCTION: Antimicrobial photodynamic therapy (APDT) is a well-known light-based technology that has been widely studied as an alternative approach to fight cutaneous leishmaniasis (CL). APDT induces lipid peroxidation in cellular membranes due to the generation of oxidative stress OBJECTIVES: In this study, we evaluated the role of 1,9-dimethylmethylene blue (DMMB)-mediated APDT on a wild-type (WT) and a miltefosine-resistant (MF) strain of Leishmania amazonesis and analyzed several cellular processes to get insights into the underlying mechanisms of APDT. MATERIALS AND METHODS: For this, APDT was carried out using red light (λ= 670±12 nm) and promastigotes were exposed to different concentrations of DMMB at 8 J/cm2. Then, we measured mitochondrial potential and intracellular levels of reactive oxygen species (ROS) and analyzed quantitative lipidomics of the main phospholipid classes using electrospray-mass spectrometry. DISCUSSION AND RESULTS: As a result, we observed overproduction of ROS, mitochondrial membrane depolarization, and a rapid lipid remodeling immediately after APDT. Of note, MF showed a higher content in levels of phosphatidylcholine (PC) as compared to the WT line before treatment, which suggests it could be also involved in the MF resistance mechanism. In addition, results showed that after APDT, PC levels were substantially decreased, while new phospholipid species of phosphatidylethanolamine (PE) were increased. CONCLUSION: In conclusion, our data suggest DMMB-mediated APDT promoted a significant lipid peroxidation in the parasite's membrane of both strains, which failed to manage redox imbalance, thus resulting in cellular malfunction and death.
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I.04 - Photochemical Characterization of Methylglyoxal

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INTRODUCTION: Methylglyoxal (MG) is a reactive electrophile α-dicarbonyl compound that is kept at low concentration in conditions of abiotic stress. Under extreme environmental conditions, the concentration of MG increases substantially, being able to react with several nucleophiles, including biomolecules with amines and cysteine and severely decreasing the antioxidant defenses. The chemical reactivity of MG could be substantially increased by the absorption of light in the UV and visible ranges; however, this was never investigated before. OBJECTIVES: Light absorption can increase the MG-related oxidative stress and we have characterized its main photophysical properties such as, quantum yields and lifetimes of singlet and triplet excited as well as singlet oxygen quantum yield (ΦΔ). MATERIALS AND METHODS: Absorption and fluorescence spectra were obtained using a Shimadzu spectrometer (UV-2400-PC) and a Varian Cary Eclipse fluorimeter, respectively. ΦΔ was determined using a NIR fluorometer (SHB) equipped with a Hamamatsu PMT, with a 400 nm LED as the excitation source. Phosphorescence was performed at 77K (liquid nitrogen temperature) in a Hitachi F-4500 fluorimeter equipped with quartz Dewar. Φf (quantum yield of fluorescence emission) and ΦΔ were obtained by measuring and comparing with a known standard and the phosphorescence lifetime by the emission decay time. DISCUSSION AND RESULTS: Absorption spectra of MG solutions indicate absorbance extending to the visible range of the spectra (~490nm). ΦΔ and Φf were 0.13 and 0.006. The presence of triplet excited state was obtained by the low temperature phosphorescence of MG, whose lifetime was 224.2 milliseconds. CONCLUSION: It was possible to confirm that MG forms long-lived excited states that can substantially increase the cytotoxicity of MG, upon exposure to UV radiation and visible light.
Keywords: Methylglyoxal, Photochemistry, Oxidative stress
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