Evaluation of methylene blue-mediated photodynamic inactivation in association with encapsulated nitric oxide donor in chitosan nanoparticles on L*eishmania (L.) amazonensis.* An in vitro study

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Cutaneous leishmaniasis (CL) is a chronic disease developed by parasites of the genus *Leishmania* that promotes destructive lesions. The available treatments are limited because of resistance and toxicity. Reactive oxygen species and nitric oxide (NO) are potentially toxic to these parasites. Photodynamic inactivation (PDI) has been explored as an alternative treatment once no reports about resistance have been described. Additionally, several studies indicate that the administration of exogenous NO donors represents an interesting strategy against CL. The aim of this work was to explore the effects of methylene blue (MB)-mediated PDI in association with encapsulated NO donors (S-nitroso-MSA) in chitosan nanoparticles (CSNPs) on *Leishmania (L.) amazonensis.* S-nitroso-MSA-CSNPs were tested with *L. (L) amazonensis* transgenic line expressing luciferase (La-LUC) at 25 μM, 50 μM, 75 μM, and 100 μM. PDI was perfomed using a red LED (λ= 660±22 nm) at fluences of 12.5, 25, 37.5 and 50 J/cm² and MB at 100 μM. The association of both therapies was performed using 25 μM of S-nitroso-MSA-CSNP immediately after PDI at 25 J/cm² fluence. Results demonstrated a 50% decrease in La-LUC metabolic activity with 25 μM S-nitroso-MSA-CSNP and a 70% reduction with 25 J/cm² fluence when the tests were performed separately. However, the association with S-nitroso-MSA-CSNP showed 97% reduction of the parasite burden.

The present study demonstrates that encapsulated S-nitroso-MSA-CSNPs were able to improve the effects of PDI on *Leishmania (L.) amazonensis*, which suggests that both therapies combined could be a potential alternative treatment for CL.