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Effects of methylene blue-mediated photodynamic inactivation associated to NO-releasing chitosan nanoparticles on cutaneous leishmaniasis in mice

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Cutaneous leishmaniasis (CL) is a chronic disease developed by parasites of the genus *Leishmania* that promotes destructive and ulcerated lesions. The available treatments are limited because of side effects, resistance and toxicity. Reactive oxygen species and nitric oxide (NO) are potentially toxic to these parasites. Photodynamic inactivation (PDI) involves the generation of oxidative stress and has been explored as an alternative treatment once it is less expensive and no reports about resistance have been described.[1,2] Additionally, several studies indicate that the administration of exogenous NO donors represents an interesting strategy against CL.[3] 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 CL in BALB/c mice using real time bioluminescence.

Promastigotes of *L. (L) amazonensis* transgenic line expressing luciferase were used. Sixteen BALB/c mice were infected in the left footpad with 1.10^6 promastigotes. After 4 weeks, mice were randomly assigned to experimental groups (n=4): Control (non-treated), PDI (treated only with PDI), PDI+CSNP (submitted to PDI and S-nitroso-MSA-CSNPs) and CSNP (treated only with S-nitroso-MSA-CSNPs). PDI was administered in two sessions separated by 24 h and CSNPs (80 □M) were applied immediately after the second PDI session. PDI was performed using a red LED (□= 660 ± 22 nm), MB (100 μM), irradiance of 100 mW/cm^2 and radiant exposure of 150 J/cm^2. Parasite burden was analyzed through luciferase detection by bioimaging in the first 96 h following treatment and every week during 4 weeks. Statistically significant differences were considered when $p < 0.05$.

Test groups presented significant reduction in parasite load compared to control during all experimental period. Twenty-four-h after treatments, parasite burden was lower for PDI+CSNP group but no statistically significant difference was observed when compared to other test groups. After 48 h, all test groups were similar. Besides, parasite load in test groups remained lower than control following 1, 2, 3 and 4 weeks post-treatment.

Under conditions used in this study, we conclude that CSNPs were not able to enhance MB-mediated PDI efficiency in *L. (L) amazonensis*-induced CL in mice.

References

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