No releasing chitosan nanoparticles associated to photodynamic therapy for *Leishmania amazonensis* inactivation. An *in vivo* study

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1. Introduction

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 investigate the effects of methylene blue (MB)-mediated PDI in association with encapsulated NO donors (S-nitroso-MSA) in chitosan nanoparticles (CSNPs) on *Leishmania amazonensis*-induced CL in mice using real time bioluminescence.

2. Study design

Promastigotes of *L. (L) amazonensis* transgenic line expressing luciferase were used. Sixteen BALB/c mice were infected in the left hind 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 μM) were applied immediately after the second PDI session. PDI was performed using a red LED (λ= 660 ± 22 nm), MB (100 μM), fluence rate of 100 mW/cm^2 and fluence of 150 J/cm². Parasite load 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 $p < 0.05$.

3.Results

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. Seven, 14 and 21 days after treatments despite lower parasite load in test groups than control, no statistically significant differences were observed. However, following 30 days test groups presented significant decrease in parasite l compared to untreated animals (Figure 1). Figure 2 displays the clinical aspect of *L. amazomensis*-induced lesions 30 days post treatments. We can notice that PDI only is able to delay ulceration. On the other hand, CSNP-treated footpads remain swelled. Both treatments show ulcerated and swelled footpads.

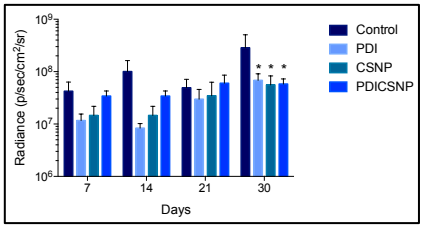

Fig.1 Parasite burden during 4 weeks post PDT

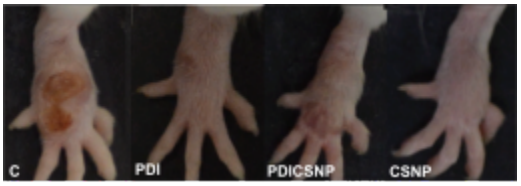

Fig.2 Clinical aspects of infected footpad 4 weeks post treatment

4. Conclusion

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.

5. References

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