

Could NO-releasing chitosan nanoparticles improve photodynamic therapy on cutaneous leishmaniasis?

Cabral, F.V.¹; Pelegrino, M.T.²; Seabra, A.B.²; Ribeiro, M.S.¹

¹Laboratory of Optical Therapy (Center of Lasers and Application/ Nuclear and Energy Research). Av.Prof.Lineu Prestes, 2242, CEP 05508-000, Brazil

²Center of Natural and Human Sciences, Universidade Federal do ABC (UFABC), Av. dos Estados 5001, CEP 09210-580, Santo Andre, SP, Brazil

Email: fe_vcabral@hotmail.com

Abstract: Photodynamic inactivation (PDI) and NO-releasing chitosan nanoparticles (CSNPs) were associated to treat cutaneous leishmaniasis in mice to verify synergism between therapies. Parasite burden, lesion size and hyperalgesia were analyzed. CSNPs were not able to improve PDI. © 2018 The Author(s)

1. Introduction

Leishmaniasis is a chronic and zoonotic disease developed by parasites of genus *leishmania*. There are two predominant forms including visceral and cutaneous manifestations whose treatments are limited because of price, toxicity and resistance. Cutaneous leishmaniasis promotes ulcerated lesions on the exposed parts of the body.¹ According to the specie and the host immune response, the disease might intensify to a more severe form that could lead to patients' death. Photodynamic inactivation (PDI) involves the generation of oxidative stress and has been studied as an alternative treatment once it is less expensive and no reports about resistance have been described.² Furthermore, literature indicates that the administration of exogenous NO donors represents an interesting strategy against CL.^{3; 4} 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.

2. Methods

Twelve BALB/c mice were infected in the left footpad with 1.10^6 promastigotes of *L. (L) amazonensis* transgenic line expressing luciferase. After 4 weeks, mice were randomly assigned to experimental groups ($n=4$): Control (non-treated), PDI (submitted to two PDI sessions), PDI+CSNP (submitted to two PDI sessions following S-nitroso-MSA-CSNPs, respectively) and CSNP (treated only with S-nitroso-MSA-CSNPs). PDI sessions were separated by 24 h and CSNPs (80 μ M) were applied immediately after. PDI was performed using a red LED ($\lambda= 660 \pm 22$ nm), MB (100 μ M), fluence rate of 100 mW/cm² and fluence of 150 J/cm². Parasite burden was analyzed through luciferase detection by bioimaging in the first 96 h following treatment and once a week during 4 weeks. Prior to imaging, mice received 75mg/kg luciferin, intraperitoneally. Disease progression was also evaluated every week for 4 weeks by measuring lesion size with a caliper and hyperalgesia with von Frey filaments. 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. In the first 24 h after treatments, parasite burden was statistically significant lower for PDI+CSNP group compared to other groups (Fig. 1a). After 48 h, all test groups were similar. Following 30 days, statistically significant differences were noticed between test groups and control (Fig 1b). Lesion size was significantly lower for all test groups compared to control (Fig. 1c). Although PDI+CSNP demonstrated a lesion size reduction, PDI group demonstrated to be clinically more effective. The association of both treatments indicated a significant hyperalgesia reduction in the first week that kept lower for the next 3 weeks (Fig 1d). However, PDI group showed a better response on nociceptive sensibility at all experimental period when compared to other groups. Figure 2 exhibits clinical appearance of CL 4 weeks after treatment.

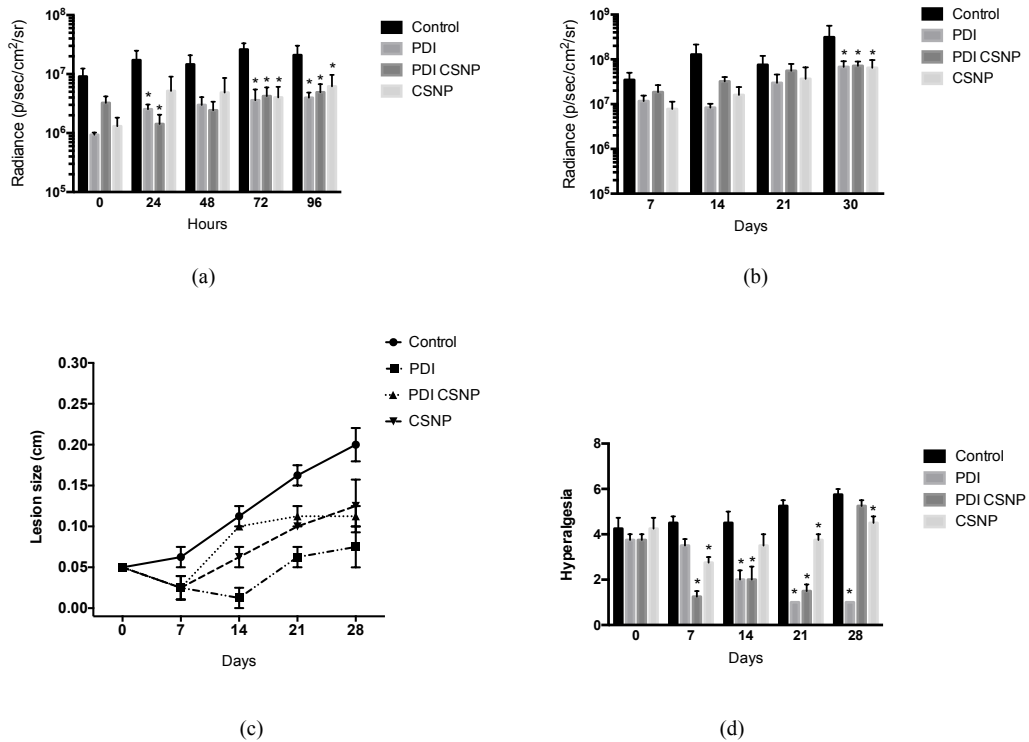


Fig. 1 Parasite burden on infected BALB/c mice in the first 96 h (a) and the next 4 weeks (b). Disease progression measured by lesion size (c) and hyperalgesia (d).

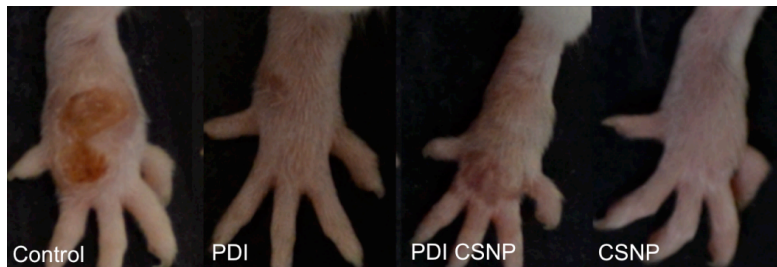


Fig. 2 Clinical appearance of experimental groups 30 days after treatments.

4. Conclusions

Under the conditions used in this study, CSNPs were not able to enhance PDI on CL in BALB/c mice.

5. References

- [1] World Health Organization (Epidemiological situation. WHO), 2016. Available: <http://www.who.int/leishmaniasis>. Accessed: (2018).
- [2] Hamblin, M. R. "Antimicrobial photodynamic inactivation: a bright new technique to kill resistant microbes." *Curr Opin Microbiol*, v. 33, p. 67-73 (2016).
- [3] Moreno, E. et al. "Nanoparticles as multifunctional devices for the topical treatment of cutaneous leishmaniasis." *Expert Opin Drug Deliv*, v. 11, p. 579-97 (2014).
- [4] De souza, G. F. et al. "Leishmanicidal activity of primary S-nitrosothiols against *Leishmania major* and *Leishmania amazonensis*: implications for the treatment of cutaneous leishmaniasis." *Nitric Oxide*, v. 15, n. 3, p. 209-16 (2006).