Pegylated nanoparticles for encapsulation of bisnaphthalimidopropyl derivatives against *Leishmania infantum*

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Alternative antileishmanial agents are an urgent need since currently available drugs for the treatment of leishmaniasis are associated with emerging resistance and elevated toxicities. Recently, bisnaphthalimidopropyl (BNIP) compounds showed promising anti-*Leishmania* activity [1,2], despite presenting some associated toxicity and low aqueous solubility, therefore claiming the use of a delivery system able to target infected tissues and increase efficiency.

The present work aimed to develop a nanoparticulate system for BNIP compounds based on PLA and PLGA polymers and evaluate the activity of the nanoformulations against the *Leishmania infantum* parasite. The effect of PEGylation on the anti-*Leishmania* activity of the nanoformulations was also assessed. BNIP-loaded nanoparticles were prepared by nanoprecipitation and PEGylation was achieved through introduction of a PLA-PEG copolymer in the preparation procedure. Physicochemical characterization of the nanoformulations included size, polydispersity index, ζ-potential, encapsulation efficiency and *in vitro* drug release determinations. The anti-*Leishmania* activity was evaluated in intracellular *L. infantum* amastigotes.

All nanoformulations presented a mean diameter in the range of 180-235 nm, low polydispersity (≤ 0.102), anionic surface charge (-1 to -7 mV) and encapsulation efficiency of BNIP compounds in the range of 80-90 %. The optimal drug loading was 5 % (w/w), since it didn't led to major disturbance of the particles characteristics and kept the encapsulation efficiency around 80 %. *In vitro* drug release studies revealed a biphasic pattern of drug release. PEGylation of nanoparticles resulted in increased release of the drugs *in vitro*; however, after 7 days of incubation, the majority of the compound stays retained in the particle matrix, in both uncoated and PEG-coated nanoparticles. Empty nanoparticles were able to inhibit the growth of intracellular amastigotes. This effect was more pronounced for uncoated nanoparticles. Based on these findings, optimization of polymer amount in the nanoformulation was carried out. For PLGA and PLA nanoparticles the maximum concentration that didn't exhibit considerable anti-*Leishmania* activity was 0.20 mg/ml, while for PLA- and PLGA-PEG nanoparticles it was of 1.00 mg/ml. Incorporation of BNIP compounds in the nanoparticles reduced the toxicity of the compounds to human and mouse macrophages by at least 10-fold. Growth inhibition assays revealed that BNIP compounds encapsulated in PEG coated nanoparticles were more effective than the free compounds in inhibiting the growth of intracellular amastigotes in human THP1 marophages.

In resume, BNIP compounds were efficiently encapsulated in uncoated and PEG-coated nanoparticles, leading to a reduction in the toxicity of the compounds on macrophage cells. Therefore, these BNIP nanoformulations tolerate the administration of higher doses because the slow and sustained release of the drug from the nanoparticles, which reduces the toxic effect associated with the administration of the free drug. PEG-coated nanoparticles containing the BNIP compounds were more effective in the growth inhibition of intracellular *L.infantum* amastigotes than free compound.

References

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Acknowledgments

S Costa Lima thanks Fundação para a Ciência e Tecnologia (FCT) for the grant SFRH/BPD/37880/2007 and Fundação Calouste Gulbenkian for the funded project P-105348.