TREATMENT OF CUTANEOUS LEISHMANIASIS WITH A SUBCUTANEOUS IMPLANT CONSISTING OF DRUG-LOADED BIODEGRADABLE NANO AND MICROPARTICLES.

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Abstract

Leishmaniasis is a neglected tropical disease that is very difficult to treat. Its cutaneous form, although not fatal as the visceral form, may develop into morbid disfiguring lesions. Despite its skin localization, conventional therapy of cutaneous leishmaniasis is based on multiple parenteral injections with systemically toxic drugs[1]. Aiming at a localized therapy for cutaneous leishmaniasis, we have used a biodegradable system for sustained subcutaneous release of an antileishmanial drug. For that, poly-(lactide-co-glycolide) PLGA particles loaded with 10 % of a novel lipophylic antileishmanial nitro chalcone CH8[2] (CH8/PLGA) were prepared by multiple emulsion and solvent evaporation methods. Particles measured in average 6 µm and had -12 mV zeta potential. When tested *in vitro* on *Leishmania amazonensis*-infected macrophages, CH8/PLGA promoted higher parasite killing than free CH8 drug, in a manner independent of macrophage activation for the production of microbicidal reactive oxygen and nitrogen reactive species. Also, CH8/PLGA was not cytotoxic to macrophages at the range of parasite-toxic concentrations. In vivo, their efficacy was tested in BALB/c mice subcutaneously infected in the ear with fluorescent *L. amazonensis*-GFP. On days 9, 16 and 23 of infection, the animals received at the infection site a subcutaneous depot injection with CH8/PLGA containing 30 µg of CH8. Controls received free CH8, empty PLGA particles, 30 µg of the reference drug Glucantime, or 10 µl of PBS vehicle alone. Treatment efficacy was monitored by measuring the ear tickeness throughout infection, and parasite loads on days 30 or 90 of infection by Limiting Dilution Assay and fluorometry. Systemic toxicity was biochemically evaluated by measuring the levels of transaminases and creatinine in the serum. Skin inflammation and implant degradation were monitored by histopathology at different times after treatment. The results showed that CH8/PLGA treatment was significantly more effective and durable than the free drug in controlling lesion and parasite growth, and in the prevention of lesion ulceration (Fig 1). A single dose with CH8/PLGA on day 9 was as effective as 3 doses with free CH8.No signs of toxicity were detected in the serum, and histopathological studies showed a transient ear inflammation on day 7 that was resolved by day 30. These findings show that PLGA nano and microparticle subcutaneous implant promoted a sustained chalcone CH8 drug release at the lesion site, with a durable and safe therapeutic effect, supporting its use for localized treatment of cutaneous leishmaniasis.

References

[1] Croft, S. L. and Olliaro, P. Clinical Microbiology and Infection, **17** (2011): 1478-1483.
[2] Boeck, P.; Falcão, C.A.B.F.; Leal, P;V.; Cechinel-Filho, V.; Torres-Santos, E.C.; Yunes, R.A. and Rossi-Bergmann, B. **14** (2006) *Bioorganic and Medicinal Chemistry*: 1538-1545.

Figure

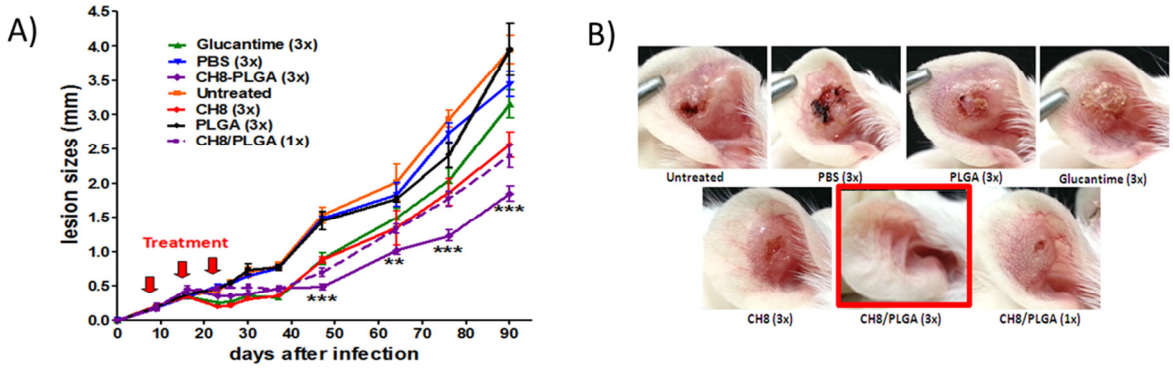

Figure1: Mice were infected with Leishmania in the ear. Then, they were given a single (1x, day 9) or 3 injections (3x, days 9, 16 and 23) with CH8/PLGA or control drugs or vehicle as indicated. A) The lesion growth with time. B) Ear appearances on day 70of infection.