Experimental Parasitology 135 (2013) 217–222

Contents lists available at ScienceDirect

Experimental Parasitology

journal homepage: www.elsevier.com/locate/yexpr

Leishmanicidal activity of amphotericin B encapsulated in PLGA–DMSA nanoparticles to treat cutaneous leishmaniasis in C57BL/6 mice

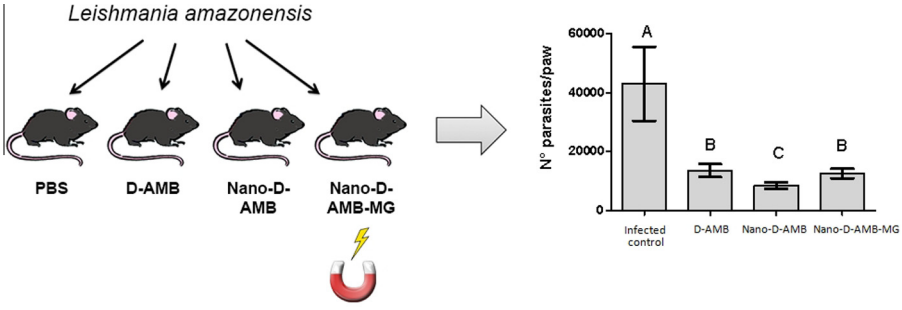
Ricardo Fontoura de Carvalho [a], Ieler Ferreira Ribeiro [b], Ana Luisa Miranda-Vilela [b,*], José de Souza Filho [b], Olímpia Paschoal Martins [d], Débora de Oliveira Cintra e Silva [c], Antônio Cláudio Tedesco [d], Zulmira Guerrero Marques Lacava [b], Sônia Nair Báo [c], Raimunda Nonata Ribeiro Sampaio [a]

[a] Faculdade de Ciências da Saúde, Laboratório de Dermatomicologia, Universidade de Brasília, 70910-900 Brasília, Brazil
[b] Instituto de Ciências Biológicas, Departamento de Genética e Morfologia, Universidade de Brasília, 70910-900 Brasília, Brazil
[c] Instituto de Ciências Biológicas, Departamento de Biologia Celular, Laboratório de Microscopia Eletrônica, Universidade de Brasília, 70910-900 Brasília, Brazil
[d] Departamento de Química, Laboratório de Fotobiologia e Fotomedicina, Centro de Nanotecnologia e Engenharia Tecidual, Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, Universidade de São Paulo, 14040-901 Ribeirão Preto, SP, Brazil

HIGHLIGHTS

- D-AMB and Nano-D-AMB had the same efficacy to reduce paw diameter of infected mice.
- Nano-D-AMB promoted a greater reduction in parasite number and cell viability.
- Nano-D-AMB favored a longer interval between drug administrations.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 13 February 2013
Received in revised form 18 June 2013
Accepted 11 July 2013
Available online 24 July 2013

Keywords:
Cutaneous leishmaniasis
Desoxycholate amphotericin B
Nanobiotechnology

ABSTRACT

The major goal of this work was to design a new nanoparticle drug delivery system for desoxycholate amphotericin B (D-AMB), based on controlled particle size, looking for the most successful release of the active agents in order to achieve the best site-specific action of the drug at the therapeutically optimal rate and dose regimen. For this, AMB nanoencapsulated in poly(lactic-co-glycolic acid) (PLGA) and dimercaptosuccinic acid (DMSA) nanoparticles (Nano-D-AMB) has been developed, and its efficacy was evaluated in the treatment of experimental cutaneous leishmaniasis in C57BL/6 mice, to test if our nano-drug delivery system could favor the reduction of the dose frequency required to achieve the same therapeutic level of free D-AMB, and so, an extended dosing interval. Magnetic citrate-coated maghemite nanoparticles were added to this nanosystem (Nano-D-AMB-MG) aiming to increase controlled release of AMB by magnetohyperthermia. Female mice ($N = 6$/group) were infected intradermally in the right footpad with promastigotes of *Leishmania amazonensis* in the metacyclic phase, receiving the following intraperitoneal treatments: 1% PBS for 10 consecutive days; D-AMB at 2 mg/kg/day for 10 days (totalizing 20 mg/kg/animal); Nano-D-AMB and Nano-D-AMB-MG at 6 mg/kg on the 1st, 4th and 7th days and at 2 mg/kg on the 10th day, also totalizing 20 mg/kg/animal by treatment end. The Nano-D-AMB-MG group was submitted to an AC magnetic field, allowing the induction of magnetohyperthermia. The evaluations were through paw diameter measurements; parasite number and cell viability were investigated by limiting dilution assay. D-AMB-coated PLGA–DMSA nanoparticles showed the same efficacy as free D-AMB to reduce paw diameter; however, the Nano-D-AMB treatment also promoted a significantly greater reduction in parasite number and cell viability compared with free D-AMB. The nano-drug AMB delivery system

* Corresponding author. Fax: +55 61 3107 2923.
E-mail addresses: mirandavilela@unb.br, analuisamv@uol.com.br (A.L. Miranda-Vilela).

0014-4894/$ - see front matter
http://dx.doi.org/10.1016/j.exppara.2013.07.008

appeared more effective than free D-AMB therapy to reduce the dose frequency required to achieve the same therapeutic level. It thus favors a longer interval between doses, as expected with development of a new nano drug delivery system, and may be useful in the treatment of many different pathologies, from cancer to neurodegenerative diseases.

1. Introduction

Leishmaniases comprise a complex group of non-contagious infectious vector-borne diseases caused by protozoa of the *Leishmania* genus, which affect more than 12 million people in 88 countries worldwide. Although its clinical forms are particularly diverse, the disease can be classified in three main forms: cutaneous leishmaniasis (CL), mucocutaneous leishmaniasis (MCL), and visceral leishmaniasis (VL) (de Souza et al., 2010; Desjeux, 2004; Launois et al., 2008; Murray et al., 2005; Wang et al., 2011; Zauli-Nascimento et al., 2010). VL is typically caused by the *Leishmania donovani* complex, which includes the species *L. donovani*, *Leishmania infantum* and *Leishmania chagasi* (Sundar and Rai, 2002; Wang et al., 2011), whereas CL and MCL forms are caused by several *Leishmania* species, including *Leishmania major*, *Leishmania tropica*, *Leishmania amazonensis* (CL) and *Leishmania braziliensis* (CL and MCL) (Minodier and Parola, 2007; Souza et al., 2011; Wang et al., 2011).

The causative species of CL determines the clinical features and courses, and the treatments (Minodier and Parola, 2007). This disease presents a broad spectrum of clinical features: it may be limited to a single part of the skin (localized cutaneous leishmaniasis) or may produce a large number of lesions (diffuse cutaneous leishmaniasis), causing severe skin lesions and, exceptionally, leading to fatal systemic infection (de Souza et al., 2010; Minodier and Parola, 2007; Nogueira and Sampaio, 2001; Silveira et al., 2009). *L. amazonensis* is clinically important in the New World, where it can cause localized CL or diffuse CL, especially in immuno-compromised hosts (de Souza et al., 2010; Zauli-Nascimento et al., 2010).

Systemic antimonials are generally required for the treatment of CL in the New World because of the risk of mucosal involvement (Minodier and Parola, 2007). This current chemotherapy has a series of limitations such as: high cost, intravenous administration and high toxicity, associated with many other undesirable side effects. The antibiotic amphotericin B (AMB), a second-choice drug, has showed good clinical results, although its use in CL treatment requires more extensive studies. However, its effectiveness is also limited and sometimes causes significant hypersensitivity reactions, nephrotoxicity, hepatotoxicity, cardiotoxicity, and other adverse effects (Launois et al., 2008; Minodier and Parola, 2007; Vyas and Gupta, 2006). Thus, the development of alternative therapies is a priority for the treatment of this infection.

The design of nano drug delivery systems for conventional drugs represents one of the most promising antimicrobial therapies due to its higher therapeutic efficacy, low toxicity, higher target delivery effect, and prolonged systemic circulation lifetime, releasing drugs in a sustained and controlled manner (Zhang et al., 2007, 2010). Moreover, because nanoformulations as a drug delivery system improve bioavailability, the protection of drugs incorporated from metabolism is a favorable feature of nanosystems, allowing prolonged drug residence in the human body, and therefore prolonging time between administrations (das Neves et al., 2010). Thus, the major goal of this work was to design a new nanoparticle drug delivery system for desoxycholate amphotericin B (D-AMB), based on controlled particle size, looking for the most successful release of the active agents in order to achieve the best site-specific action of the drug at the therapeutically optimal rate and dose regimen. For this, AMB nanoencapsulated in poly(lactic-co-glycolic acid) (PLGA) and dimercaptosuccinic acid (DMSA) nanoparticles (Nano-D-AMB) has been developed, and its efficacy was evaluated in the treatment of experimental cutaneous leishmaniasis in C57BL/6 mice, to test if our nano-drug delivery system could favor the reduction of the dose frequency required to achieve the same therapeutic level of free D-AMB, and so, an extended dosing interval. Because hyperthermia based on magnetic nanoparticles results in controlled release of the drug (Kumar and Mohammad, 2011), magnetic citrate-coated maghemite nanoparticles were added in this nanosystem (Nano-D-AMB-MG), in an attempt to increase the controlled release of AMB by magnetohyperthermia.

2. Material and methods

2.1. Syntheses of nanostructured D-AMB samples

The polylactic acid (PLA), polyglycolic acid (PGA), DMSA and the D-AMB used to prepare Nano-D-AMB were purchased from Sigma (St Louis, MO, USA). The sample of Nano-D-AMB was prepared according to Amaral et al. (2009), with slight modification. The polymers (50 mg of PLA and 50 mg of PGA) were first dissolved in 10 ml of dichloromethane. This organic solution received the addition of 120 mg of D-AMB and 0.05 M DMSA as an additive. To another solution of 40 mL of a phosphate buffer saline solution (PBS) containing polyvinyl alcohol (PVA) 1%, was added the initial organic solution of PLA-PGA with vigorous agitation in a blender operating ultra turrax system (10,000 rpm) to obtain the initial water–in-oil emulsification. The organic solvent was removed from the solution by stirring at room temperature and evaporation under reduced pressure. The nanoparticles were centrifuged (25 °C, 5000 rpm) in intervals of 10 min. The preparation was washed three times in distilled water, suspended in 1.0 mL physiological PBS solution, and stored at 4 °C. All procedures were developed in a sterile room with all the manipulation in sterile wood. The stability of the suspension was analyzed over time and maintained for 3 weeks. The process was protected by a patent deposited in the INPI (National Institute of Intellectual Property, Brazil), PI # 0700446-0.

Magnetic D-AMB polymer sample (Nano-D-AMB-MG) was developed following the same protocol described above. The magnetic fluid sample based on citrate-coated maghemite nanoparticles was synthesized and characterized as previously described (da Silva et al., 2003), with maghemite nanoparticles being obtained via oxidation of magnetite nanoparticles by co-precipitation of Fe (II) and Fe (III) ions in alkaline medium (Soler et al., 2004). The precise volume of the stock solution of citrate-coated maghemite nanoparticles at 2.3×10^{16} particles/mL was added to the initial phosphate buffer saline solution (PBS) containing polyvinyl alcohol (PVA 1%). To this solution was finally added the organic phase containing D-AMB and the DMSA additive with vigorous agitation in a blender operating ultra turrax system (10,000 rpm) to obtain the initial water–in-oil emulsification. The final isolation of the Magnetic D-AMB sample was identical to the procedure described above for the non-magnetic one, rendering a stabilized fluid with 7.8 ± 2.6 nm average-diameter maghemite nanoparticles coated with citrate, at the concentration of 3.4×10^{13} particles/mL, used in the experiments.