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Poloxamer 407 (Pluronic® F127)-based polymeric micelles for amphotericin B: *In vitro* biological activity, toxicity and *in vivo* therapeutic efficacy against murine tegumentary leishmaniasis

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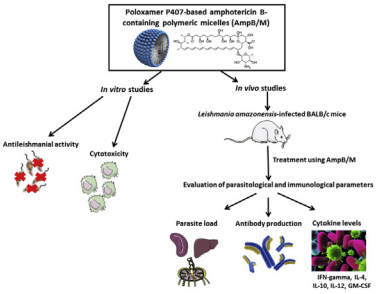
HIGHLIGHTS

- Poloxamer 407-based amphotericin B-containing micelles (AmpB/M) were developed.
- AmpB/M showed satisfactory anti-leishmanial activity and selectivity index.
- AmpB/M was also shown to be effective in treating *Leishmania amazonensis*-infected BALB/c mice.

GRAPHICAL ABSTRACT



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ABSTRACT

In the present study, a Poloxamer 407-based amphotericin B (AmpB)-containing polymeric micelles system (AmpB/M) was employed in the treatment of *Leishmania amazonensis*-infected BALB/c mice. Initially, the *in vitro* antileishmanial activity (IC_{50} value) of AmpB/M and B-AmpB/M (empty micelles) against stationary promastigotes and amastigotes-like forms of the parasites was determined, and results were of 1.83 ± 0.4 and 22.1 ± 0.7 μM, respectively, for the promastigotes, and of 2.27 ± 0.5 and 33.98 ± 2.6 μM, respectively, for the amastigotes-like. The cytotoxic concentration (CC_{50}) values of these products were also evaluated, and we found the results of 119.5 ± 9.6 and 134.7 ± 10.3 μM, respectively. With these values, the selectivity index (SI) was calculated and results were of 65.3 and 5.4, respectively,

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for the promastigotes, and of 59.3 and 3.96, respectively, for the amastigotes-like of the parasites. Free AmpB showed IC_{50} values of 1.2 ± 0.3 and 2.5 ± 0.5 μM for the promastigotes and amastigotes-like, respectively, whereas the CC_{50} value was of 9.5 ± 0.4 μM. The SI values of this drug were of 7.9 and 3.8, respectively, for the promastigote and amastigote-like stages of the parasites. After, animals were infected and received saline or were treated subcutaneously with free AmpB, AmpB/M or B-AmpB/M. In the results, free AmpB-treated and infected mice showed reductions in their body weight, which were associated with hepatic and renal damage; however, no organic alteration was observed in the AmpB/M-treated animals. In addition, these animals showed significant reductions in their lesion average size and in the parasite burden in all evaluated infected tissue and organs, when compared to the other groups; as well as significantly higher levels of antileishmanial IFN-γ, IL-12, GM-CSF and nitrite, which were associated with low production of IL-4, IL-10 and IgG1 isotype antibodies. In conclusion, this AmpB/M system could be considered as an alternative for future studies in the treatment of tegumentary leishmaniasis.

1. Introduction

Leishmaniasis affects about 12 million people in 98 countries worldwide (Alvar et al., 2012). The severity of the disease depends primarily of the parasites infective species and of the immune response generated by the infected mammalian hosts (Kaye and Scott, 2011). Tegumentary leishmaniasis (TL) is the most common form of leishmaniasis, presenting an incidence of about 1.2–1.5 million new cases each year (WHO, 2010). This disease is endemic in more than 70 countries, where 90% of the cases have been registered in Afghanistan, Algeria, Brazil, Pakistan, Peru, Saudi Arabia and Syria (Desjeux, 2004). The disease exhibits distinct clinical manifestations such as cutaneous leishmaniasis (CL), diffuse cutaneous leishmaniasis (DCL) and mucosal leishmaniasis (ML) (Grimaldi and Tesh, 1993; Reithinger et al., 2007). In Brazil, TL is caused mainly by infection with *Leishmania (Viannia) braziliensis, Leishmania (V.) guyanensis* and *Leishmania (Leishmania) amazonensis* species (Marzochi and Marzochi, 1994; Silveira et al., 2004). *Leishmania amazonensis* presents particular importance, since it is able to cause a broad spectrum of clinical manifestations in the infected hosts, ranging from cutaneous to visceral leishmaniasis (Barral et al., 1991; Garcez et al., 2002).

The current treatment for leishmaniasis is still based on the administration of pentavalent antimonials; however, their use presents problems such as the requirement of intramuscular or intravenous injections, as well as the occurrence of side effects such as anorexia, myalgia, arthralgia, pancreatitis, leukopenia, besides of renal, hepatic and cardiac disorders in the patients (Vyas and Gupta, 2006; Minodier and Parola, 2007). Pentamidine, miltefosine and paramomycin have been applied as alternatives drugs, but their use is also limited due to the high toxicity (Chawla and Madhubala, 2010). Since the drug discovery is a long and very costly process, requiring an investment of more than $1.0 billion to identify, characterize and develop new pharmaceuticals (Hughes et al., 2011), the research for new delivery systems to carry out conventional drugs, with the purpose to reduce their toxicity, but without losing their biological activity, could be considered relevant (Neves et al., 2010; Zhang et al., 2007, 2010; Carvalho et al., 2013; Ribeiro et al., 2014).

Amphotericin B (AmpB) has been shown to present an effective *in vitro* antileishmanial activity against different *Leishmania* species, such as *Leishmania infantum, Leishmania braziliensis* and *L. amazonensis* (Ordóñez-Gutiérrez et al., 2007; Chávez-Fumagalli et al., 2015; Cunha et al., 2015; Duarte et al., 2016). However, its clinical use is also hampered due to the toxicity in the patients (Croft and Coombs, 2003; Vyas and Gupta, 2006). As a consequence, lipid-based formulations carrying AmpB have been formulated with the purpose of reducing the toxicity of this drug (Bern et al., 2006; Rosenthal et al., 2009; Ribeiro et al., 2014). The development of these formulations made it possible to solve this problem, and the World Health Organization has recommended the use of liposomal AmpB for the treatment of visceral leishmaniasis (VL) (WHO, 2010). However, the main restrictions against the widespread use of these formulations have been based on their intravenous application and high cost (Chávez-Fumagalli et al., 2015).

In this context, the search for obtaining new delivery systems that can be used for the treatment of leishmaniasis continues. In the present study, a Poloxamer P407 (Pluronic® F127)-based AmpB-containing polymeric micelles system (namely AmpB/M) was developed and evaluated *in vitro* and *in vivo* against *L. amazonensis*. Firstly, the *in vitro* antileishmanial activity of the free AmpB (as a drug control), AmpB/M and B-AmpB/M (as a delivery control) was evaluated against stationary-phase promastigote and amastigote-like stages of the parasites, aiming to establish their *Leishmania* minimum inhibitory concentrations (IC_{50}), as well as their cytotoxic effects on murine macrophages (CC_{50}). Secondly, the formed compounds were employed in the treatment of TL, by evaluating their therapeutic efficacy to treat *L. amazonensis*-infected BALB/c mice. Experiments were developed to evaluate the parasite load in the infected tissue and organs of the treated and infected animals, as well as to investigate the immune response generated after the treatments performed, by investigating the cytokine production, the humoral response and the nitrite secretion in these animals.

2. Materials and methods

2.1. Mice

Female BALB/c mice (8 weeks age) were obtained from the breeding facilities of the Department of Biochemistry and Immunology, Institute of Biological Sciences, Federal University of Minas Gerais (UFMG), Belo Horizonte, Minas Gerais, Brazil. Animals were maintained under pathogen-free conditions. The study was performed in compliance with the National Guidelines of the Institutional Animal Care and approved by Committee for the Ethical Handling of Research Animals (CEUA) from UFMG (protocol number 182/2012).

2.2. Parasites

L. amazonensis (IFLA/BR/1967/PH-8) strain was used. Parasites were grown in complete Schneider's medium (Sigma, St. Louis, MO, USA), which was composed by Schneider' s medium supplemented with 20% heat-inactivated fetal bovine serum (FBS, Sigma) and