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Note

Formulation of amphotericin B as nanosuspension for oral administration

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Abstract

Amphotherin B was formulated in a nanosuspension as a new oral drug delivery system for the treatment of experimental visceral leishmaniasis. Amphotericin B (AmB) nanosuspensions were produced by high pressure homogenisation obtaining particles with a PCS diameter of 528 nm. Environmental stability was determined in artificial gastrointestinal fluids at different pH and electrolyte concentrations. In vivo efficacy was determined in a mouse model of visceral leishmaniasis. Following oral administration (5 mg kg^{-1}), micronised amphotericin B did not show any curative effect. However, administrations of amphotericin B nanosuspension, reduced liver parasite load by 28.6% compared to untreated controls.

Keywords: Amphotericin; *Leishmania*; Nanosuspension; Oral

Increasing the oral bioavailability of poorly soluble drugs is a major goal when seeking to improve the activity of both experimental and clinically established drugs. Antiparasitic drugs like amphotericin B (AmB), artemisinin and atovaquone (halofantrine) have limitations in clinical administration due to their poor solubility and other unfavourable properties. Moreover, intravenous injection and infusion can be associated with considerable fluctuation of drug concentrations in the blood and serious side effects like nephrotoxicity, for example with AmB (Müller et al., 2000).

Because of its poor solubility, AmB is not absorbed in the gastrointestinal tract (GIT). To overcome this problem AmB is used parenterally as liposomal (AmBisome®) or as colloidal dispersion (Fungizone®, Abelcet®) for the treatment of systemic *Candida* infection or for the treatment of visceral leishmaniasis (Arikan and Rex, 2001). Nevertheless, with a background of high costs, low compliance and technical problems with administration in endemic countries, an oral delivery system is highly desirable. Oral delivery could improve patients' compliance and pharmacokinetics of a drug. Modern approaches to increasing oral absorption include micronisation (e.g. pearl milling), polymeric nano- and micro-particles, cyclodextrines (Müller et al., 2000; Müller and Peters, 1998), and, more recently, formulation as cochleates (Santangelo et al., 2000).

The aim of this study was, to employ the nanosuspension technique to produce AmB nano-particles

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for oral administration, thereby avoiding the use of harmful additives and enabling controlled, prolonged drug delivery when treating visceral leishmaniasis in Balb/c mice. As described earlier (Kayser, 2001; Müller and Peters, 1998), nanosuspensions show adhesion to the gastrointestinal mucosa, prolonging the contact time of the drug and thereby enhancing its uptake via the GIT.

Nanosuspensions of AmB were produced by a high pressure homogenisation technique using a Micron LAB 40 homogeniser (APV Systems, Unna, Germany). Amphotericin B (Sigma–Aldrich, Germany) was suspended at a concentration of 0.4% (m/m) in an aqueous solution of Tween 80 (0.5% m/m), Pluronic F68 (0.25% m/m), and sodium cholate (0.05% m/m). The pre-suspensions were dispersed using an Ultra Turrax for 5 min at 9500 rpm. This coarse pre-suspension was homogenised at 150 and 500 bar for two cycles each, then at 1500 bar for 15 cycles. The size reduction process resulted in a suspension in the nanometer range characterised by photon correlation spectroscopy (PCS) and laser diffractometry (LD). As these nanosuspensions were intended for oral administration there was no need to further verify particle size or to strictly exclude all particles over 5 μm.

Long term stability was determined over a period of 21 days. The results, depicted in Table 1, indicated no increase in particle size (LD d99% = 0.624 μm) and showed a zeta potential of −36 mV indicating high stability of the product at 20 °C. The electrolyte and pH stability of the nanosuspensions was tested in artificial gastrointestinal fluids at pH 7.4, 3.5 and 1.1. From neutral to acid pH an increase of the PCS diameter from 0.712 to 0.985 and 1.156 μm, respectively, was observed. In parallel, the LD d99%-values increased from 4.21 to 5.96 and 9.21 μm.

Table 1
Laser diffractometry (LD) and photon correlation spectroscopy (PCS) diameter, polydispersity index (PI) and zeta potential of amphotericin B nanosuspension after production (Day 0) and after 21 days of storage at 20 °C (Day 21)

Parameter	Day 0	Day 21
LD d50%	0.680 μm	0.197 μm
LD d95%	0.607 μm	0.494 μm
LD d99%	0.690 μm	0.624 μm
PCS diameter	0.528 μm	0.495 μm
PI	0.28	0.29
Zeta potential	−38 mV	−36 mV

Drug absorption and reduction was determined in an in vivo mouse model using female Balb/c mice with an average weight of 18–23 g. The procedure is described in detail by Croft and Yardley, 1999. Briefly, mice were infected i.v. with 1×10^7 *Leishmania donovani* strain MHOM/ET/67/L82 amastigotes. Treatment began on Day 7 post infection (p.i.). All compounds were given orally by feeding with a blunt syringe at a dose level of 5 mg kg^{-1} BW for 4 or 5 consecutive days. Mice were sacrificed Day 14 p.i., livers were then weighed and impression smears prepared. The smears were fixed in methanol and stained with Giemsa's stain (10%) for 45 min. The number of *Leishmania* amastigotes per liver cell nuclei was determined microscopically and calculated as percent of the mean values of untreated controls.

As shown in Fig. 1, neither oral administration of micronised AmB, Ambisome® nor of Fungizone® significantly reduced liver parasite load compared to untreated controls. However, when the AmB nanosuspension was administered, a significant reduction ($P < 0.5\%$) of the parasite load by 28.6% was observed, clearly indicating a superior oral uptake and systemic efficacy of the formulation as nanosuspension.

In conclusion; this is the first report on the in vivo antiparasitic activity of amphotericin B formulated as a nanosuspension for oral administration. Whereas all

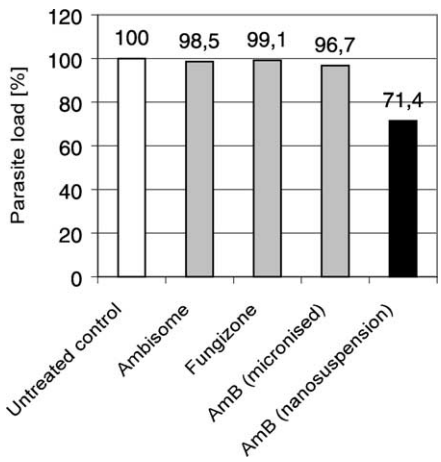

Fig. 1. Percentage reduction of *Leishmania donovani* parasite load in livers of infected Balb/c mice

other drugs and formulations tested; liposomal AmB (Ambisome®), micronised AmB and Fungizone®, did not show any curative effect at all upon oral administration, the oral AmB nanosuspension significantly reduced parasite numbers. Furthermore, AmB nanosuspension proved to be stable over at least 3 weeks, indicating good shelf life characteristics. Stability in high electrolyte and acid pH environment is still a problem that needs to be solved in the future in order to further improve oral efficacy. With respect to pharmaco-economics we present a cost effective drug delivery system for AmB that is easy to prepare and has potential for further development.

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