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Atovaquone and rifabutine-loaded nanocapsules: formulation studies

F. Dalençon [a], Y. Amjaud [a], C. Lafforgue [a], F. Derouin [b], H. Fessi [a,*]

[a] *Laboratoire de Recherche et Developpement en Pharmacie Galénique Industrielle, ISPB, Université Lyon-1, 8, avenue Rockefeller, 69008 Lyon, France*

[b] *Laboratoire de Parasitologie-Mycologie, Hôpital Saint-Louis, 1 avenue Claude Vellefaux, 75475 Paris cedex 10, France*

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Abstract

Atovaquone and rifabutine have potential therapeutic activity against toxoplasmosis but the low water solubility of these drugs reduces their bioavailability. Their formulation as a colloidal suspension of poly (D,L) lactic acid nanocapsules can increase that effectiveness. The atovaquone formulation was more stable than the rifabutine one and was injected at 15 mg/kg per day by the intragastric route to mice infected with *Toxoplasma gondii*. A better survival rate was observed in mice treated with nanocapsules than in mice treated with a suspension of the drug. Parasites were undetectable in brains of mice treated with atovaquone nanocapsules one month previously whereas control mice treated with drug suspension showed signs of central nervous system infection. © 1997 Elsevier Science B.V.

Keywords: Toxoplasmosis; *Toxoplasma gondii*; Rifabutine; Atovaquone; Nanocapsules

1. Introduction

Toxoplasmosis is a common opportunistic infection in patients with AIDS and the protozoan parasite, *Toxoplasma gondii*, causes congenital infections in children of women who acquired primary toxoplasmic infections during pregnancy (Remington et al., 1990). In primary infections in immunodeficient patients (Porter and Sande, 1992), *Toxoplasma gondii* is in the tachyzoite form (Araujo et al., 1991) producing lysis of infected cells. Following this first step, the parasite is transformed in bradyzoites, tissue cysts in the central nervous system. Drugs such as atovaquone and rifabutine (Kovacs, 1992; Araujo et al., 1994) exhibit, alone or in combination, an in vitro and in vivo activity against *Toxoplasma gondii*.

The potential of colloidal drug carriers in targeted and controlled delivery of antiparasitic

* Corresponding author.

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compounds has received much interest. Moreover, nanocapsules represent a promising approach to oral delivery which might lead to increase bioavailability of rifabutine and atovaquone. Previous work has shown that administration of atovaquone with a fatty meal resulted in enhanced therapeutic effect (Rolan et al., 1994), by increasing its solubility in the gut lumen, and the formulation of atovaquone and rifabutine in an aqueous suspension of nanocapsules can increase their intestinal absorption.

2. Materials and methods

Polymeric nanocapsules were prepared by a modification of the method described by Fessi et al. (1989); 125 mg of polymer, poly D,L lactide (PLA) molecular weight 88 000 (Boeringer Ingelheim, France) was dissolved in 25 ml of acetone under magnetic stirring. After dissolving 100 mg of surfactant, (Epikuron 170®, Lucas Meyer, Hambourg, Germany) in the organic phase, drug solubilized in 0.5 ml of oil was added. The combined organic phase was finally poured into 50 ml of an aqueous solution of the hydrophilic surfactant (Synperonic® PE/F68, 100 mg, ICI, Clamart, France) under moderate magnetic stirring. The acetone was evaporated under reduced pressure.

Nanocapsule mean size was determined by using a nanosizer N4 (Coulter Co, Harpenden, UK). Incorporated drug was assayed by HPLC, using a Hewlett Packard 1050 system with a Lichrospher 100 RP C18 125×4 mm reverse phase column.

Atovaquone samples were eluted in an acetonitrile/water/glacial acetic acid (85:15:5 v/v) mixture at constant flow rate of 3.0 ml/min, detection was carried out at 254 nm. Rifabutine was detected at 275 nm and the mobile phase consisted of acetonitrile/KH_2PO_4 0.5 mol/l (60:80 v/v).

3. Results and discussion

Atovaquone and rifabutine solubility studies in various oils yielded better results with benzyl benzoate compared with Miglyol® or Labrafac® or oleic acid (Table 1). The limits of solubility were 24.2 mg/ml for atovaquone and 78.4 mg/ml for rifabutine. Consequently benzyl benzoate was used in the formulation. The maximal drug concentrations in the nanocapsules evaluated were 1200 μg/ml for atovaquone and 3920 μg/ml for rifabutine.

Table 1
Atovaquone and rifabutine solubility in oils used for nanocapsule formulations

	Atovaquone (mg/ml)	Rifabutine (mg/ml)
Miglyol 812®	7.4	81.0l
Benzyl benzoate	24.2	78.4
Labrafac Hydro®	7.5	84.2
Oleic acid	<7.4	—

Stability studies for atovaquone show that for concentrations ranging from 200 to 1000 μg/ml, the yield of encapsulation was always greater than 84%. One hundred percent of the drug was encapsulated because atovaquone has a very low solubility in water ($< 2.10^{-4}$ mg/ml). The size of atovaquone nanocapsules was measured for each concentration and over a period of 6 months for the 1000 μg/ml concentration (Fig. 1). The evolution of size may be explained by polymer degradation.

A similar analysis of rifabutine formulations at two concentrations (1600 and 4000 μg/ml) showed very high encapsulation yields (93% for 1600 μg/ml and 86% for 4000 μg/ml). Size evalua-

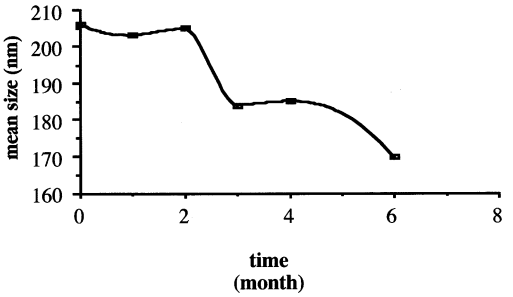

Fig. 1. Atovaquone nanocapsules mean size determinated by quasi-elastic light analysis diffusion during a period of six months.

Table 2
effect of atovaqune nanocapsules versus atovaquone suspension on parasitic loads in blood brain and lung homogenates of mice infected with *T. gondii*

	No treatment	Unloaded nanocapsules	Atovaquone suspension	Atovaquone nanocapsules
Blood	4.1	5.01	0	0
Brain	5.75	5.33	3.82	0
Lung	>6.87	>6.83	>6.18	1.9

Results in arbitrary units.

tion showed good results for the first concentration (size: 205 nm and polydispersity: 2). However with concentrations as high as 4000 μg/ml, the nanocapsules were larger and had a high polydispersity (512 nm, polydispersity: 5).

The suspension of atovaquone nanocapsules appeared to be the most promising. The instability of rifabutine could be explained by the relative solubility of its ionized form in water and the pH of the preparation, increasing rifabutine migration from the nanocapsule oily core to the aqueous medium.

Only atovaquone nanocapsules were used for the in vivo experiments.

Experiments were carried out in mice (Swiss OF1, IFFA CREDO, l'Arbresle, France) infected with *Toxoplasma gondii* COUL Strain, type II wich produces tissue cysts. Atovaquone formulations, as a suspension (particles from 200 to 500 μm) or as nanocapsules (210 nm), were injected at 15 mg/kg per day by the intragastric route for 10 days. Untreated mice infected with 10^4 tachyzoites died between the 5th and the 7th day.

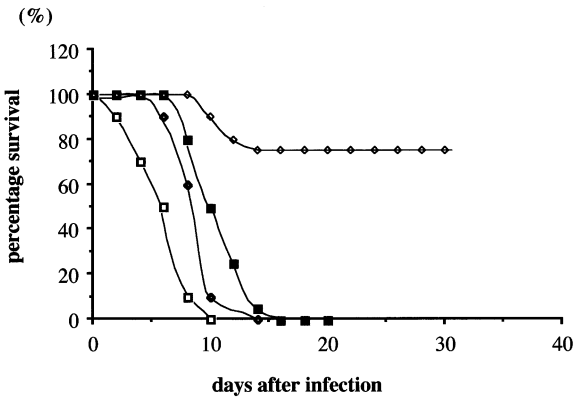

Fig. 2. Percentage survival of mice infected with *Toxoplasma gondii*: without any treatment (□), treated with unloaded nanocapsules (◆), with atovaquone suspension (■) and with atovaquone-loaded nanocapsules (◇).

Antiparasitic atovaquone activity was evaluated by quantification of parasites in blood, lungs and brain of infected mice (Table 2) according to a method described elsewhere (Piketty et al., 1990) and by determination of percentage survival (Fig. 2). Parasitic loads were determined by subculturing dilutions of blood brain and lung homogenates on fibroblast tissue cultures. Demonstration of *T. gondii* was performed by an indirect immunofluorescence assay using a rabbit anti-*T. gondii* antibody and a fluorescein labelled anti-rabbit immunoglobulin G conjugate.

Results in mice infected with *Toxoplasma gondii* have shown good results when atovaquone nanocapsules were injected by the intragastric route. A dramatic increase of therapeutic effects of this formulation compared with the suspension was observed in terms of brain bradyzoites and percentage survival.

Although these drugs have potential activity against *Toxoplasma gondii* infection, their low solubility reduces bioavailability. Nanocapsule formulations are a promising alternate formulation to suspensions. It is particularly interesting, that the atovaquone formulation employing benzyl benzoate as the oily phase could also be envisaged for parenteral administration where it would also be expected to yield good results.

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