Evaluation of the paromomycin loading characteristics in nanoprecipitated PLGA nanospheres

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ABSTRACT

Cryptosporidium parvum is a spore forming protozoan parasite, an important endopathogen in humans' biliary ducts inducing chronic infection. Paromomycin exhibits an activity against *C. parvum.* Unfortunatly, it has poor oral bioavailability and is responsible of ototoxicity and nephrotoxicity. The aim of this work was to optimize the preparation of PLGA nanoparticles loaded with paromomycin by a nanoprecipitation technique. The influences of the amount of polymer and the volume of the organic phase on the characteristics of the nanospheres were assessed. Physico-chemical characterization of the loaded nanoparticles was performed. Unimodal, 290nm sized, spherical nanoparticles were obtained, with a low encapsulation of 3%. DSC thermograms did not show interaction between PLGA and paromomycin. Despite the simplicity of the process, no evidence of incorporation of the drug was obtained. These investigations revealed that other drug carrier system must be explored.

Keywords: *C. parvum*, paromomycin, PLGA, nanoparticles, drug carrier,

1 INTRODUCTION

Cryptosporidiosis is a widely distributed enteric disease of humans and animals caused by *Cryptosporidium parvum*. It is a spore forming protozoan parasite that produces highly infectious and resistant oocysts, and it is considered as one of the most important endopathogens in humans [1]. This parasite is well recognised as a cause of intestinal infection in various mammalian species [2]. In healthy humans, cryptosporidiosis may produce self-limiting diarrhea [3,4,5], whereas in immunocompromized individuals, especially in AIDS patients, the diarrhoea is often severe, prolonged and may even persist throughout life.

At present, there is no reliable chemotherapy or chemoprophylaxis for cryptosporidiosis in humans or animals and the disease has very limited treatment options despite the testing of numerous antimicrobial agents. Anticryptosporidial pharmacological and/or immunological agents were initially tested in immunodeficient models of cryptosporidiosis. Sinefungin, paromomycin, and pentamidine dimethanesulfone are among the few agents with partial or demonstrable activity against C. parvum [6,7,8,9].

Paromomycin is a natural aminoglycoside to be effective in the treatment of cryptosporidiosis in patients with AIDS [10] and to inhibit *Cryptosporidium* infection of a human enterocyte cell-line [11] and is currently used as the first-line agent against crytosporidial infection in AIDS patients6.

In a model of immunodepressed rats, it has been demonstrated that the biliary ducts are the main site of the parasite development inducing chronic infection [12]. Paromomycin exhibits an activity against *Cryptosporidium parvum* for a bile concentration higher than 400 µg/ml [13]. Unfortunatly, paromomycin has poor oral bioavailability and is responsible of ototoxicity and nephrotoxicity. The encapsulation of the paromomycin into nanoparticulate carrier may be an opportunity for improve the drug targeting and consequently to reduce the administered doses and the side-effects.

The aim of this work was to optimize the preparation of poly(lactic-co-glycolic acid) (PLGA) nanoparticles loaded with paromomycin by a nanoprecipitation technique. PLGA was chosen because of its biodegradability and biocompatibility features [14]. In order to improve the delivery system, especially the drugs loading efficiency, the influences of the amount of polymer and the volume of the organic phase on the characteristics of the nanospheres were assessed. Physico-chemical characterization of the loaded nanoparticles was performed.

2 MATERIAL AND METHODS

2.1 Material

Paromomycin sulphate was purchased from Antbioticos (Milan, Italy). Poly(DL-lactic-co-glycolic acid) (PLGA, 50:50) was supplied by Boehringer Ingelheim (Ingelheim, Germany). Poloxamer 188 (Synperonic PE/F-68) was purchased from ICI (Kortenberg, Belgium). The HPLC mobile phase reagent (PIC® B-8 Low UV reagent) was obtained from Waters (Saint-Quentin, France). All other chemicals were of analytical grade.

2.2 Preparation of nanoparticles

The paromomycin-loaded nanoparticles were prepared according to a modified nanoprecipitation method [15]. The starting procedure was as follows. PLGA polymer (100mg) was dissolved in acetone (30ml). This organic phase was then poured in a constantly magnetic stirred aqueous phase (40ml) of paromomycin (10mg) and 0.5% (w/v) of synperonic poloxamer, a stabilizing agent (10mg). The mixture was kept under stirring until the acetone evaporated, and the nanoparticle dispersion were then condensed to the desired final volume of 10ml, in a rotavapory evaporator (Rotavapor® Büchi RE 140) and filtered through a 0,2µm pore sized filter (Millex, Millipore). Drug-free nanoparticles were prepared according the same procedure omitting the drug.

To investigate the influence of various formulation parameters on drug incorporation efficiency, the following alterations were made to the starting procedure: To assess the effect of the quantity of polymer, 100mg polymer was replaced by 75, 150, 225, 300and 450mg. To study the influence of the volume of acetone, 30ml was replaced by 20ml. The influence of these two factors was also assessed on the nanosphere production yield and the paromomycin encapsulation efficiency.

2.3 Determination of drug incorporation efficiency

The encapsulation efficiency was determined by difference between the total amount of paromomycin in the final suspension and the free drug present in the aqueous suspending medium following separation of nanoparticles by centrifugation at 18000 rpm for 60 minutes (Beckman Centrifugator J2 211 M/E). The paromomycin was assayed by HPLC analysis using a Beckman 110A system with detection at 200 nm. The separation was achieved using a Macherey-Nagel Nucleosil® C8 column (100 Å; 150 x 4.6 mm i.d.). The mobile phase adjusted at pH 4.4 was a mixture of methanol and aqueous solution of PIC® B-8 Low UV reagent (52/48 %). The samples were eluted at a constant flow rate of 1ml/min and assayed at 200 nm. Preliminary experiments have determined the retention time of paromomycin to be 4 minutes.

2.4 Physico-chemical characterization

Particle size: The average diameter of the nanoparticle was measured by Photon Correlation Spectroscopy (PCS) using a Coulter® N4MD submicron particle analyzer (Coultronics, Margency, France). A monochromatic laser light ray was used. The analysis was performed at a scattering angle of 90° and at a temperature of 25°C. The samples were appropriately diluted with filtered water (0,2 µm filter Millipore).

Nanoparticle morphology: The morphology of the paromomycin loaded nanoparticles was analysed using Transmission Electron Microscopy (Tecnai 12 Biotwin, FEI Company). Carbon-coated copper grids and uranyle acetate 1% as a negative stain were used. The samples were examined with the electron microscope set at 120kV.

Differential scanning calorimetry (DSC): The thermal analysis of paromomycin powder, PLGA powder, paromomycin-loaded nanoparticles, and the physical mixture of paromomycin powder with PLGA powder were performed. The thermograms were recorded with a DSC-6 differential scanning calorimeter (DSC-6, Perkin Elmer) calibrated with indium and zinc. The thermal behaviour was studied by heating 5.0±2.0mg of the samples in a hermetically sealed aluminium pan. The temperature range used was between 30 and 200°C, with a heating rate of 10°C/minute under a nitrogen atmosphere (flow rate 30 ml/minute) using an empty aluminium pan as the reference.

3 RESULTS AND DISCUSSION

Results of the influence of the formulation parameters studied (amount of polymer and volume of organic phase) on the nanoparticles characteristics (mean particle size and encapsulation efficiency) are as follows.

The influence of the amount of polymer on the mean particle size is shown on figure 1. When the amount of polymer varies from 75 to 300 mg, the average size of the nanospheres slightly increases up to 290 nm with a unimodal size distribution. In contrast, the use of a higher amount of polymer (450 mg) resulted in a bimodal size distribution with a dramatically increased mean particle size (490 nm). This effect is explained by the formation of large agregate of polymer during the nanoprecipitation. As reported by P. Couvreur [16], one of the most critical conditions for obtaining nanoparticles by this technique without any bulk precipitation of the raw material was the low polymer concentration in the organic phase.

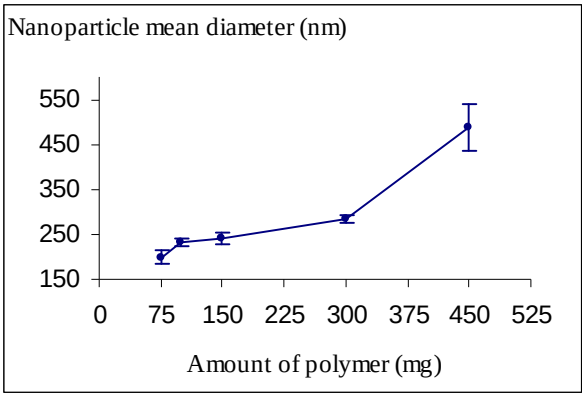

Figure 1. Influence of the amount of polymer on the mean particle size (n=3).

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The influence of the volume of acetone on the mean particle size was also studied. When the volume of acetone was reduced to 20ml, the mean particle size was significantly higher in comparison with that obtained with 30ml acetone. This is probably due to the increasing concentration of the polymer in the organic phase involving a more rapidly precipitation of the nanoparticles in the aqueous phase.

The influence of these two factors was also assessed on the nanosphere production yield (Figure 2) and the paromomycin encapsulation efficiency (Figure 3). The encapsulation efficiency was in all cases below 3% and the investigated parameters showed no significant influence on the loading efficiency. Similar results were previously observed for other drugs, including indomethacin (2.0% or 5.8%), dexamethasone (0.9%), itraconazole (4.1%) and sinefungin (9.18%) [17,18,19]. This was probably due to the hydrophilic nature of the drug which diffuses in the aqueous phase during the nanospheres formation. Furthermore, no evident interaction occurred between the hydrophobic PLGA used and the drug. Only slight absorption with very low strength may be observed and the centrifugation of the particles during the drug loading determination was enough to induce desorption of the paromomycin. Despite the simplicity of the process used, it seemed difficult to enhance the encapsulation efficiency of hydrophilic compounds. For instance, Niwa observed low drug encapsulation efficiency for fluroro-uracile and for nafarelin [20,21]. These investigations revealed that other drug carrier system must be explored to improve further the drug encapsulation efficiency of the carrier system. This would allow the reduction of the quantity of carrier required for drug administration to the target site.

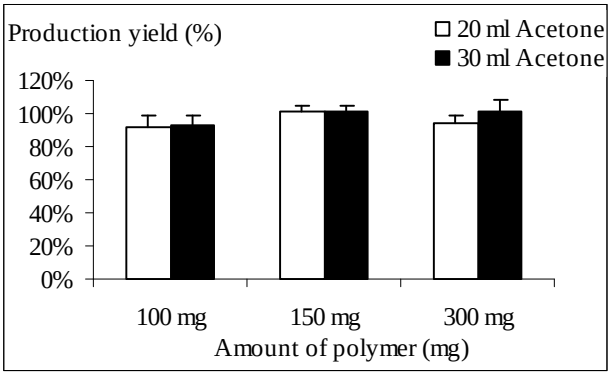

Figure 2. Influence of the volume of acetone on the production yield (n=3).

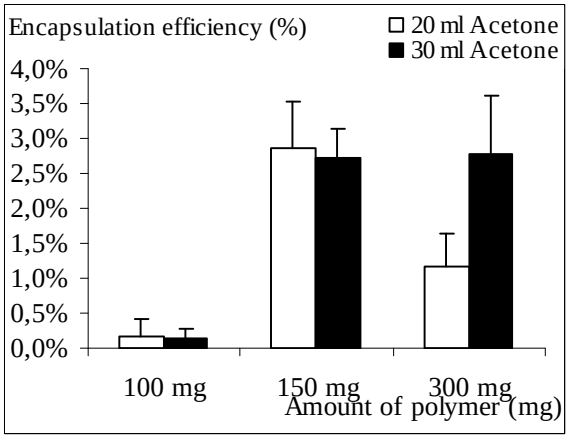

Figure 3. Influence of the volume of acetone on encapsulation efficiency (n=3).

The DSC thermograms of paromomycin, PLGA, paromomycin-PLGA physical mixture, and paromomycin-PLGA nanoparticles were determined (Figure 4). The thermogram of the paromomycin showed an endothermic peak at 179.64°C corresponding to the melting temperature of the drug. The peak at 49.18°C corresponded to the melting temperature of the polymer PLGA. The thermogram of the physical mixture showed the same melting peak of the drug and the polymer without modification. In contrast, in the DSC thermogram of paromomycin-loaded PLGA nanoparticles did not show new entity. The endothermic peak of paromomycin disappeared and the melting temperature of PLGA was reduced. This result conforms that no evident interaction occurred between the PLGA and the drug. The centrifugation of the particles induced probably the desorption of the paromomycin.

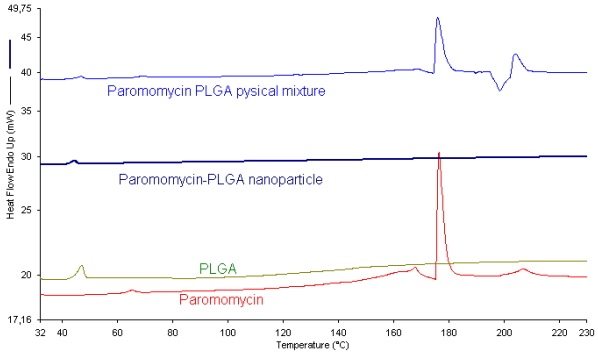

Figure 4 : Thermograms of PLGA 50:50 powder, paromomycin powder, a physical mixture of paromomycin and PLGA, and paromomycin-loaded PLGA nanoparticles.

4. CONCLUSION

Various PLAGA nanospheres formulations were prepared in order to achieve the encapsulation of paromomycin sulfate. No evidence of incorporation of the drug was obtained and a more hydrophilic polymer and appropriate method of manufacture could be tested in order to obtain nanospheres carrier for their evaluation in animals infected with Cryptosporidium parvum.

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