International Journal of Pharmaceutics 444 (2013) 10–17

Contents lists available at SciVerse ScienceDirect

International Journal of Pharmaceutics

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

Pharmaceutical nanotechnology

In vivo/in vitro pharmacokinetic and pharmacodynamic study of spray-dried poly-(DL-lactic-*co*-glycolic) acid nanoparticles encapsulating rifampicin and isoniazid

L.L.I.J. Booysen[a,b], L. Kalombo[a], E. Brooks[c], R. Hansen[d], J. Gilliland[c], V. Gruppo[c], P. Lungenhofer[d], B. Semete-Makokotlela[a], H.S. Swai[a], A.F. Kotze[b], A. Lenaerts[c], L.H. du Plessis[b,*]

[a] *Council for Scientific and Industrial Research, Pretoria 0001, South Africa*
[b] *Unit for Drug Research and Development, North-West University, Potchefstroom Campus, Potchefstroom 2520, South Africa*
[c] *Department of Microbiology, Immunology and Pathology, Colorado State University, Fort Collins, CO 80523-4629, USA*
[d] *Department of Pharmacology, CSU Animal Cancer Centre, Fort Collins, CO 80523-4629, USA*

ARTICLE INFO

Article history:
Received 29 October 2012
Received in revised form 14 January 2013
Accepted 18 January 2013
Available online 26 January 2013

Keywords:
PLGA nanoparticles
In vitro
In vivo
Pharmacokinetic
Pharmacodynamic
Rifampicin
Isoniazid
PEG coated

ABSTRACT

Poly-(DL-lactic-*co*-glycolic) acid (PLGA) nanoparticles were prepared by a double emulsion solvent evaporation spray-drying technique and coated with polyethylene glycol (PEG 1% v/v). The PLGA nanoparticles had a small size (229 ± 7.6 to 382 ± 23.9 nm), uniform size distribution and positive zeta potential (+12.45 ± 4.53 mV). *In vitro*/*in vivo* assays were performed to evaluate the pharmacokinetic (PK) and pharmacodynamic (PD) performance of these nanoparticles following nanoencapsulation of the anti-tuberculosis drugs rifampicin (RIF) and isoniazid (INH). The results demonstrated the potential for the reduction in protein binding of these drugs by protection in the polymer core. Furthermore, *in vitro* efficacy was demonstrated using *Mycobacterium tuberculosis* (*M. tb.*) (strain $H_{37}Rv$). Sustained drug release over seven days were observed for these drugs following once-off oral administration in mice with subsequent drug distribution of up to 10 days in the liver and lungs for RIF and INH, respectively. It was concluded by these studies combined with our previous reports that spray-dried PLGA nanoparticles demonstrate potential for the improvement of tuberculosis chemotherapy by nanoencapsulation of anti-tuberculosis drugs.

© 2013 Elsevier B.V. All rights reserved.

1. Introduction

The World Health Organization (WHO) reported in 2011 that the global tuberculosis (TB) incident cases have fallen since 2006. However, because 8.8 million new TB incident cases in were reported in 2010, the TB burden is still considered a global crisis. In 2010, approximately 10 million children were orphaned as a result of parent deaths caused by TB (WHO, 2012). South Africa currently ranks third of the 22 high burden countries in terms of TB incident cases per 100,000 population. Limitations exist in TB chemotherapy such as non-localised delivery of drugs, high dose and dose frequency as well as the adverse side effects that the therapy presents. To address these challenges various groups have reported the encapsulation of antituberculosis drugs where slow release, improved intracellular delivery and high drug loading parameters can be achieved by nanoencapsulation (Ahmad et al., 2006, 2008; Azarmi et al., 2008; Kisich et al., 2007). Furthermore, nanoencapsulation of drugs in a biodegradable polymer has been reported to minimize first pass metabolism *via* protection of the drug in the core of the polymeric shell (Couvreur and Vauthier, 2006). Physico-chemical properties of nanoparticles such as size, surface charge, hydrophobicity and polymer composition all contribute toward protein binding, biodistribution, cellular uptake and immune response. Poly-(DL-lactic-*co*-glycolic) acid (PLGA) is a biodegradable and biocompatible polymer widely used in pharmaceutical applications (Jain, 2000). Liposomal drug carrier technology also has numerous advantages in drug delivery and have demonstrated improved efficacy in cancer chemotherapy (Ledet and Mandal, 2012). However, a few drawbacks exist for this system such as leakage of the drug, limited shelf-life, instability *in vivo*, difficulties in optimization of the surface structure and limitation to the parenteral use only (Epstein et al., 2008).

This report aims to evaluate the *in vitro*/*in vivo* properties of spray-dried PLGA nanoparticles for the purpose of sustained drug delivery and distribution of the anti-TB drugs rifampicin (RIF) and isoniazid (INH). Our previous reports have demonstrated the protein binding, biodistribution, macrophage uptake and immune

* Corresponding author. Tel.: +27 018 299 4246; fax: +27 018 299 2248.
E-mail address: Lissinda.DuPlessis@nwu.ac.za (L.H. du Plessis).

0378-5173/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.ijpharm.2013.01.038

response to these nanoparticles in the absence of encapsulated drug (Semete et al., 2010a,b, 2012). This report will show the potential of the nanoparticles in TB chemotherapy, by demonstrating *in vitro* efficacy and sustained drug release and drug distribution *in vivo* following once-off oral dosing. RIF and INH was selected based on the fact that they are two of the four first-line drugs in TB chemotherapy and the most widely investigated (Benator et al., 2002; Brooks and Orme, 1998; Dhillon and Mitchison, 1992; Ellard, 1999; Grosset and Leventis, 1983; Gurumurthy et al., 1999; Maggi et al., 1966; Panchagnula and Agrawal, 2004; Pape et al., 1993; Takayama et al., 1972).

2. Methods

2.1. Nanoparticle preparation

Nanoparticles were prepared with PLGA 50:50 (Mw: 45,000–75,000) using a modified double emulsion solvent evaporation spray-drying technique (Kalombo, 2008) as described in Semete et al. (2012). PLGA (100 mg) dissolved in ethyl acetate were added to phosphate buffered saline (pH7.4) and emulsified with a high speed homogeniser with varying speed between 3000 and 5000 rpm. The water-in-oil (w/o) emulsion was added to 1% (w/v) polyvinyl alcohol (PVA) and the mixture was further emulsified at 8000 rpm. The double emulsion (w/o/w) was spray dried at 95–110 °C with an atomising pressure between 5 and 8 bars. The most important variation in the formulation is the encapsulation of drug. RIF was added in the oil phase with the polymer and INH was added in the aqueous phase. For polyethylene glycol (PEG) coating, a 40 ml mixture was prepared consisting of 5 ml of 1% v/v PEG, 10 ml of 5% lactose, 15 ml of 1% v/v PVA and 10 ml of 0.3% chitosan during the second emulsion step. The first w/o emulsion was then dispersed in this mixture and emulsified. The second w/o/w emulsion was then spray dried.

2.2. Particle characterization

Particle size and size distribution of PLGA and zinc oxide (ZnO) particles as well as polystyrene beads were measured by Dynamic Laser Scattering (DLS), also referred to as Photon Correlation Spectroscopy (PCS), using a Malvern Zetasizer Nano ZS (Malvern Instruments Ltd., UK). Nanoparticles (1–3 mg) were suspended in filtered water (0.2 μm filter), then vortexed and/or sonicated for a few minutes. The zeta potential was also determined using the same instrument. Surface morphology of PLGA nanoparticles was analysed *via* scanning electron microscopy (LEO 1525 Field Emission Scanning Electron Microscope). Preparation of the samples for scanning electron microscopy analysis was done by means of the gold sputtering technique. The nanoparticles were fixed to the aluminium sample stubs with double sided carbon tape and sputter coating with gold was applied for viewing by scanning electron microscopy. Encapsulation efficacy was determined spectophotometrically. Nanoparticles (10 mg) were washed in 20 ml of deionised water and the particles were collected by centrifugation. Free (unencapsulated) drug in the supernatant was analysed and quantified at absorbance wavelengths for RIF (330 nm) and INH (262 nm). The amount of drug analysed was calculated against the amount of drug in the original formulation. The percentage drug loading is calculated using the amount of drug in sample calculated from the encapsulation efficiency (EE%) against the final yield of the batch. The following equations were used to determine the encapsulation efficiency and drug loading:

$$\text{Encapsulation efficiency (\%)} = \frac{\text{amount of drug in sample}}{\text{amount of drug in formulation}} \times 100 \quad (1)$$

$$\text{Drug loading} = \frac{\text{amount of drug sample}}{\text{yield (mg)}} \times 100 \quad (2)$$

2.3. In vitro protein binding assays

The nanoparticle protein binding was analysed using an adapted method as described previously for protein adsorption to polymer nanoparticles (Stolnik et al., 2001; Semete et al., 2012). In short, to determine the percentage protein binding of the unencapsulated (free) drugs as positive controls, equilibrium dialysis was used. Free RIF and INH controls were prepared in the same ratios with human plasma as the nanoparticle formulations and placed in the sample chamber of an equilibrium dialysis device with buffer chamber containing PBS (pH 7.4). Diffusion against a concentration gradient was facilitated on an orbital shaker at 100 rpm for 4 h at room temperature. The samples in both chambers were analysed using UV-spectrophotometry at 330 nm for RIF and 262 nm for INH. The samples collected from the sample and buffer chambers were analysed to determine the percentage of unbound drug. Percentage unbound drug was calculated as illustrated in Eq. (3).

$$\left(\frac{\text{drug PBS}}{\text{drug Plasma}} \times 100\right) = \%\text{unbound}$$
$$\%\,\text{bound} = 100\% - \%\text{unbound} \quad (3)$$

The remainder of the pellet solution was analysed *via* sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) gel for visual analysis using resolving and stacking gels (6%). The SDS-PAGE gels were prepared in 80 mm × 70 mm × 1.5 mm casting chambers. The percentage crosslink selected was based on obtaining adequate separation of the different size protein fragments. Separation of 30 μl of samples was performed at a constant voltage of 200 V on a Bio-Rad Power-Pac™. Protein fragments were stained with Page Blue Stain™ for 1 h and destained overnight with deionized water. The ChemiDoc XRS Plus gel dock system from Bio-Rad was used for visual documentation of the gels.

2.4. Animals used in assays

For the *in vivo* experiments specific pathogen-free, immunocompetent female Balb/C mice 6–8 weeks old were acquired from Charles River Laboratories, Wilmington, MA. The mice weighed 18–23 g and were housed under standard environment conditions at ambient temperature of 25 °C, and supplied with food and water *ad libitum*. Ethics approval was obtained for this study from the Colorado State University's Institutional Animal Care and Use Committee (IACUC), Fort Collins, CA.

2.5. Culture of Mycobacterium tuberculosis (M. tb)

M. tb (strain H37Rv, Trudeau Institute, Saranac Lake, NY) was grown in 50 ml of 7H9 broth (Difco) containing oleic acid-albumin-dextrose-catalase (OADC) enrichment (7H9-OADC) and 0.05% Tween 80. The cultures were incubated at 37 °C with rotary agitation, grown to mid-exponential phase (optical density at 600 nm [OD_{600}] of approximately 0.6–0.8, at 14–21 days), and harvested by centrifugation. The cell pellets were resuspended in a small amount of the enriched 7H9-OADC medium containing 10% sterile glycerol, transferred to cryogenic vials, and stored at −70 °C as starter stocks for further use. To prepare stock for an experiment, starter stock was added to 50 ml 7H9-OADC containing 0.1% Tween 80 and incubated at 37 °C with agitation. The starter culture was grown to an OD_{600} of 0.3–0.5 and then diluted to an OD_{600} of approximately 0.1 by using 7H9-OADC containing 0.1% Tween 80 (resulting in ~3×10^5 CFU per well). OD_{600} readings were measured spectrophotometrically (BioRad Benchmark Plus).

Download English Version:

https://daneshyari.com/en/article/2502505

Download Persian Version:

https://daneshyari.com/article/2502505

Daneshyari.com