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Pharmacokinetics, tissue distribution and relative bioavailability of isoniazid-solid lipid nanoparticles

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ABSTRACT

Low levels of isoniazid gain access into plasma following oral administration due to its high aqueous solubility, poor permeability and rapid and extensive hepatic metabolism. Further, a small $t_{1/2}$ of 1–4 h indicates its short stay in plasma and the need for repetitive or high doses which may subsequently result in hepatotoxicity and neurotoxicity associated with its use. Isoniazid-solid lipid nanoparticles (SLNs) were prepared to achieve improved bioavailability and prolonged effect, thus minimizing pulsatile plasma concentrations (and associated side effects at peak plasma concentrations). Developed SLNs showed high entrapment efficiency (69%) and small size (d_{90} 48.4 nm) such that they are expected to bypass reticulo-endothelial system (RES) pickup resulting in prolonged circulation times and since liver is the major site of metabolism of isoniazid, RES avoidance will reduce its elimination from the body. Single dose (25 mg/kg BW) oral pharmacokinetic studies were performed in plasma and various tissues of rats. A significant improvement ($p < 0.001$) in relative bioavailability in plasma (6 times) and brain (4 times) was observed after administration of isoniazid-SLNs with respect to the free drug solution at the same dose. Insignificant changes in liver concentration coupled with bypass of first pass metabolism and slow release of isoniazid (60%, in 24 h) indicate low incidence of hepatotoxicity. Isoniazid-SLNs showed a 3 times higher LD50.

1. Introduction

Isoniazid belongs to the class of nucleoside reverse transcriptase inhibitor and is the most important drug among the five ATD (antitubercular drug) recommended by WHO (World Health Organization) for the management of all forms of tuberculosis. Isoniazid is having the highest aqueous solubility among all the first line ATD (230 mg/ml at 25 °C, determined as per OECD TG 105). The low permeability (Mariappan and Singh, 2003) (log *P* of −0.402 at 25 °C, determined as per OECD TG 107) makes isoniazid a potential candidate for delivery via lipid based nanoparticulate system as this would not only improve gut permeability but may also show a lymphatic uptake and an avoidance of hepatic first pass metabolism of isoniazid. This will reduce both the therapeutic dose and the incidence of dose dependent side effects associated with long term use of isoniazid. These majorly include hepatotoxicity and neurotoxicity (Desai and Agarwal, 2004; Maryam et al., 2010; Metushi et al., 2011; Tostmann et al., 2008).

Numerous efforts are made by the scientific community to tackle the problems of tubercular treatment (Duncan and Barry, 2004; Goldman and Laughon, 2009). New drug development approaches are being tried upon but no better agent than the present first line agents has been made so far since the discovery of rifampicin in 1963 (Anon., 2010). Efforts are also being made to improve the current drug therapy by the use of novel drug delivery systems with an aim of improving the bioavailability. Polymeric nanoparticulate systems are reported for isoniazid and are shown to exhibit promising results and encourage the use of these systems over the age old delivery approaches like the tablets, capsules and liquid orals (Pandey and Khuller, 2006; Pandey et al., 2003, 2006). However, the polymeric nanoparticles are having their own problems like the costly raw material and the method of preparation which usually involves organic solvents. Though these polymeric nanoparticles have opened up a door to the management of tuberculosis in a different way however, the use of these polymeric particles are challenging especially when the therapy is long enough and during which carrier mediated toxicity like the one arising from the use of organic solvents in preparation of polymeric nanoparticles could not be avoided (Gohla and Dingler, 2001; Kante et al., 1982; Limayem et al., 2004).

In contrast, solid lipid nanoparticles (SLNs) have received considerable interest due to their ability to overcome the limitations of previous colloidal carriers (Kang et al., 2005; Kaur et al., 2008; Lee et al., 2007; Schwarz et al., 1994; Yang et al., 2009) and offer an alternative to the polymeric nanoparticles (Jenning et al., 2002). They are supposed to be identical to oil/water emulsion for parenteral nutrition, but the liquid lipid of the emulsion has been replaced by the solid lipid (Cavalli et al., 2000). SLNs can be prepared from

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biodegradable and non toxic ingredients like fatty acids, mono, di and triglycerides and phospholipids, which are normal constituents of the human body and are thus biocompatible (Almeida et al., 1997; Rawat et al., 2008; Westesen et al., 1997). SLNs can efficiently incorporate lipophilic drugs (Hu et al., 2004; Lim et al., 2004; Tabatt et al., 2004) because the latter can be incorporated easily within the lipidic core. However, encapsulation of hydrophilic materials into the hydrophobic matrix of SLNs is a major challenge as the drug tends to partition toward the aqueous phase during the production process (Cortesi et al., 2002). There are limited examples of hydrophilic drugs being encapsulated into SLNs (Jie et al., 2007; Morel et al., 1996; Nair et al., 2011; Olbrich et al., 2004; Reithmeier et al., 2001; Singh et al., 2010a). Very recently, a method to prepare solid lipid nanoparticles of hydrophilic drugs with improved entrapment efficiency was reported by us (Kaur and Bhandari, 2012a,b). In the present study, we explored the potential of isoniazid-solid lipid nanoparticle (isoniazid-SLN) in improving the oral bioavailability of isoniazid.

2. Materials and methods

2.1. Materials

Isoniazid was obtained as a gift sample from Panacea Biotec Ltd., Lalru, Punjab, India. Soy lecithin (Phospholipon 90H) was received as a gift sample from Lipoid GmbH, Germany. Compritol 888 ATO® was a gift sample from Colorcon Asia Pacific Pvt. Ltd., India. Stearic acid and Tween 80 were purchased from Central Drug House, Mumbai. Other chemicals and solvents used were of analytical or HPLC grade.

2.2. Methods

2.2.1. Preparation and characterization of SLN

Solid lipid nanoparticles of isoniazid were prepared using a recently patented method (Kaur and Bhandari, 2012a,b; Bhandari and Kaur, 2012a). Briefly the lipidic phase (lipid-8%) and the aqueous phase (polysorbate 80, soy lecithin and water) were heated to ~10 °C above the lipid melt temperature. The proportion of surfactant and the volumes of two phases were so adjusted that a microemulsion was formed spontaneously upon mixing the two phases. Hot microemulsion, thus formed was transferred into cold water (~2 °C) under constant stirring (WiseTis HG-15 D, 10,000 rpm) to obtain SLNs. The prepared SLNs were used as such for further studies.

2.2.2. Particle size, PDI and zeta potential

SLN formulation was characterized for particle size, PDI and zeta potential using DelsaNano C, Beckman Coulter, Inc. TDW was used as a dispersant medium.

2.2.3. Particle shape and surface morphology

Transmission electron microscopy (TEM) analysis of the prepared SLNs was carried out to study the morphology like sphericity and aggregation. Sample was examined by TEM (H 100, Hitachi Ltd., Japan). Samples were stained with phosphotungstic acid (PTA, 2%, 5 min and excess PTA removed), spread on a gold grid and examined.

2.2.4. Total drug content (TDC) and entrapment efficiency (EE)

Total amount of drug per unit volume present in the formulation was determined by suitably disrupting 0.1 ml of the SLN dispersion in 5 ml chloroform:methanol (2:1) volumetrically. The amount of isoniazid was determined by HPLC. Each experiment was performed in triplicate. A similarly processed blank SLN sample was taken as control. The total drug content was determined by using the equation given below.

$$\text{TDC} = \frac{\text{calculated amount of drug/ml of SLN dispersion}}{\text{total amount of drug added/ml of SLN dispersion}} \times 100 \quad (1)$$

EE was determined by analyzing the clear supernatant obtained by centrifuging the developed SLN dispersions at 8.02 lakh *g* for 2 h at 4 °C using Beckman Coulter Ultracentrifuge (L100K and 100 Ti rotor). The EE was calculated as follows:

$$\text{EE} = \frac{\text{TDC} - D_f}{\text{TDC}} \times 100 \quad (2)$$

where D_f = amount of drug in clear supernatant fluid.

2.3. In vitro release studies

In vitro release was determined in pH 6.8 phosphate buffer by dialysis bag method using dialysis membrane with a molecular weight cut off of 12,000–14,000 Da. An accurate volume (1 ml) of dispersion containing 2.6 mg of isoniazid (as per the calculated TDC) was placed inside the dialysis bag, tied at both the ends and dipped in the dissolution medium (80 ml). The solubility of isoniazid in the dissolution medium was 220 mg/ml at 25 °C. Stirring was maintained at 100 rpm, using magnetic bead, at 37 ± 0.2 °C. Two milliliters aliquots were withdrawn at pre-set time intervals (0.25, 0.5, 1, 2, 3, 4, 5, 8, 12, 24 h) and replaced by an equal volume of fresh dissolution medium. After suitable dilution, the samples were analyzed by HPLC to avoid excipient interference (i.e. to avoid overlapping of isoniazid (λ_{max} = 261 nm) and Tween 80 (λ_{max} = 244 nm)). Corrected concentration of isoniazid was calculated in the test samples using the regression equation of the calibration curve.

2.4. Pharmacokinetic studies

2.4.1. Study design

For in vivo pharmacokinetic studies, female Wistar rats weighing 160–180 g were used. The protocol was duly approved by the Institutional Animal Ethics Committee (IAEC) of Panjab University, Chandigarh, India. The animals were divided into two groups (n = 6). Groups I and II were administered 25 mg/kg BW (Lounis et al., 2001) of isoniazid either as a solution of the free drug (group I) or as isoniazid-SLN (group II) orally using an oral feeding cannula. The blood samples (0.5 ml) were withdrawn from sinus under clavicle and, collected into heparinized microcentrifuge tubes (containing heparin equivalent to 50 μL per ml blood (Higgins, 2007)) at different time intervals. Plasma was separated by centrifuging the blood samples at 4000 rpm for 10 min at 4 °C. After centrifugation, the plasma obtained was stored at −20 °C until analysis. The animals were sacrificed thereafter and the drug concentration in different organs (brain, liver and kidney) was determined after homogenizing the organs in ice-cold 150 mM KCl solution before analysis, using a previously validated HPLC method (Bhandari and Kaur, 2012b).

2.5. Sample preparation

2.5.1. Plasma

To 150-μL aliquot of plasma, 300 μL of the deproteinizing agent (methanol) was added and the dispersion was vortexed for 2 min. The samples were then centrifuged at 15,000 rpm for 10 min at 4 °C. The supernatant was collected and an equal volume of water (to increase polarity in order to obtain higher limits of quantifications) was added to the clear supernatant. The samples were then filtered (0.20 μm nylon filters) and were injected into the HPLC system.