International Journal of Pharmaceutics 455 (2013) 229–234

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

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

Pharmaceutical nanotechnology

Evaluation of novel lipid based formulation of β-Artemether and Lumefantrine in murine malaria model

Sushant Patil[a], Shital Suryavanshi[b], Sulabha Pathak[b], Shobhona Sharma[b], Vandana Patravale[a,*]

[a] Department of Pharmaceutical Sciences and Technology, Institute of Chemical Technology, N.P. Marg, Matunga, Mumbai 400019, India
[b] Molecular Parasitology Lab, B 332, Department of Biological Sciences, Tata Institute of Fundamental Research, Colaba, Mumbai 400005, India

ARTICLE INFO

Article history:
Received 4 May 2013
Received in revised form 5 July 2013
Accepted 8 July 2013
Available online 22 July 2013

Keywords:
Lipid
Artemether
Lumefantrine
Parasitemia
Survival
Recrudescence

ABSTRACT

The present investigation aims at formulating lipid based drug delivery system of β-Artemether and Lumefantrine and comparative pharmacological evaluation with innovator formulation. Commercial modified oil and indigenous natural fatty acids comprised the oily phase in developing lipidic formulation of β-Artemether and Lumefantrine. The developed system was characterized for mean globule size, stability by freeze thaw cycles, and birefringence. Developed formulation and innovator formulation were compared for their *in vivo* anti-malarial activity at different dose levels in male Swiss mice, infected with lethal ANKA strain of *Plasmodium berghei*. The percent parasitemia, activity against time and animal survival period were examined.

On fourth day of antimalarial studies, at normal and ½ dose levels, formulations revealed zero percent parasitemia while control showed 33.92 ± 6.00% parasitemia. At 1/10 dose level, developed and innovator formulations revealed zero percent parasitemia upto 11th day, however, three mice from innovator formulation demonstrated recrudescence after 12th day. Both the formulations at normal dose and ½ dose levels showed 100% activity and survival whereas at 1/10 dose level, innovator formulation showed, 62.5% survival. The developed lipidic system of β-Artemether and Lumefantrine exhibited excellent antimalarial activity with 100% survival.

© 2013 Elsevier B.V. All rights reserved.

1. Introduction

Malaria is the most intimidating vector-borne infectious disease that is prevalent in tropical and subtropical regions (Carballeira, 2008). The most important reason for this alarming situation is the non judicious use of the existing antimalarial moieties leading to the emergence of resistant parasites. Several approaches have been explored toward preventing the rampant resistance development by the parasite (Fischer and Bialek, 2002; Snow et al., 2001).

Antimalarial combination therapy which has been widely explored by the research scientists involves simultaneous use of two or more blood schizontocidal drugs with independent modes of action against distinct biochemical targets in the parasite. Among the developed combinations, WHO recommend few rational combinations and β-Artemether (BAT)–Lumefantrine (LFT) is one of them (Olumese, 2006). The rationale for combining these two antimalarials with different modes of action was to couple the synergistic fast onset of action of BAT with the long duration of action of LFT. BAT, is essential for rapid clearance of parasitaemia and rapid resolution of symptoms. It reduces parasite numbers by a factor of approximately 10,000 in each asexual cycle, which is more than other current antimalarials (which reduce parasite numbers 100–1000-fold per cycle). BAT is effective against drug resistant malaria and additionally it reduces gametocyte carriage (Lefèvre and Thomsen, 1999). However the drug exhibits a short half-life of 2–3 h (Mordi et al., 1997). This drawback is taken care of by combining it with LFT which acts slowly and has a longer half-life (Ezzet et al., 1998). This long-acting effect of LFT is thought to prevent recrudescence (Lefèvre et al., 2001). BAT and LFT together help to reduce the selective pressure on the parasite to develop resistance. In presence of fatty meal, LFT shows erratic absorption and significantly increased bioavailability (approximately 16-fold). In case of BAT, bioavailability increases by 2-fold (White et al., 1999).

BAT is a Biopharmaceutics Classification System (BCS) Class II drug exhibiting low aqueous solubility with higher permeability and also gets quickly metabolized in the gastrointestinal tract (GIT), while LFT has low solubility and low permeability (BCS Class IV) (Lindenberg et al., 2004). Thus, primary challenge is to design an oral formulation which not only enhances the solubility of both the drugs but also overcomes the metabolism of BAT in the GIT with enhanced permeability of LFT. To overcome these

* Corresponding author. Tel.: +91 22 33612217; fax: +91 22 33611020.
E-mail addresses: vbp_muict@yahoo.co.in, vb.patravale@ictmumbai.edu.in (V. Patravale).

0378-5173/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.ijpharm.2013.07.033

biopharmaceutical challenges, versatile formulation approaches which will retain the physicochemical properties of the individual drugs while simultaneously overcoming the physiological challenges are required (O'Driscoll and Griffin, 2008). Lipid based drug delivery systems have been demonstrated to be useful in enhancing the bioavailability of such BCS Class II molecules. Since these lipids based excipients keep the drug in the dissolved state until it is absorbed, they overcome the barrier of slow dissolution rates (Hauss, 2007; Dressman et al., 1998). Lipids are one of the most versatile excipient classes currently available, providing the formulator with many potential options for improving and controlling the absorption of poorly water-soluble drugs (Pouton and Porter, 2008).

Thus, the present work is focused on development of lipid based drug delivery systems of BAT and LFT in combination to increase the solubility of both the drugs, which could probably facilitate the absorption of drugs and overcome the present drawback of inconsistent bioavailability.

2. Materials and methods

2.1. Materials

BAT was provided as a gift sample by Ipca Laboratories Ltd. (Mumbai, India). LFT was provided as a gift sample by Macleod Pharmaceuticals (Mumbai, India). Capmul® MCM (Medium Chain Mono- and Diglycerides) was provide as gift sample from Abitech Corporation (Mumbai, India). Sesame oil, sunflower oil and cotton seed oil were gifts from Kamani Oil Industries Ltd. (Mumbai, India); Cremophor® EL (PEG-35-hydrogenated castor oil) and Solutol HS 15 (PEG-660-12-hydroxystearate) were gifts from BASF India Ltd. (Mumbai, India). Colloidal silicondioxide was gift sample from Evonik Degussa India Private Limited. Neusilin® (Magnesium Aluminometasilicate) was gift sample Fuji Chemical Industry Co. Ltd Japan. Acetonitrile (HPLC grade), Tween® 80, oleic acid, lactose; all other chemicals and solvents were purchased from s. d. Fine Chemicals Ltd., Mumbai, India. Double distilled water was used throughout the experiment whenever necessary.

2.2. Parasite

Among murine models of *Plasmodium*, *P. berghei* ANKA strain was used for evaluation of antimalarial activity. This strain was examined and found to be free of contamination with *Eperythrozoon coccoides*. This strain is well characterized in Tata Institute of Fundamental Research (TIFR) and is known to provide high mortality in mice, providing a good model to estimate survival and antimalarial efficacy in reducing parasitemia. It is sensitive to all currently used antimalarial drugs.

2.3. Animals

In bred (TIFR) male Swiss albino mice which were an *eperythrozoon*-free and aged 6–7 weeks. Each group contained 8 mice, having body weight in the range of 30 ± 5 g. The animals were quarantined in the animal house maintained at $22 \pm 3\,^{\circ}$C and 65% relative humidity. 12 h dark/light cycle was maintained throughout the experiments and animals were fed a standard mouse diet and provided with clean drinking water *ad libitum* throughout the experiments. The protocol was approved by the Institutional Animal Ethical Committee of TIFR [TIFR/IAEC/2009-3, Approval–07/08/09]. Experiments were carried out according to the CPCSEA (Committee for the Purpose of the Control and Supervision on Experiments on Animals) guidelines. Mice were infected by intraperitoneal inoculation of donor mouse blood diluted in ACD (acid citrate dextrose) containing approximately 10^6 infected red blood cells (RBCs) on day '0'. Formulations were administered to mice by oral gavage.

2.4. HPLC analysis of Artemether and Lumefantrine

HPLC method was developed for the analysis of drugs. The system consisted of Jasco PU-2080 plus Intelligent HPLC pump (Jasco, Japan) equipped with a Jasco UV 2075 Intelligent UV/VIS detector (Jasco, Japan), Rheodyne 7725 injector (Rheodyne, USA), Crompass Chromatography Software (Version) integrator software. The analysis was performed at 214 nm with Hi Q Sil (reversed phase C18, 4.6 mm × 250 mm and 10 μm particle size) maintained at $25 \pm 3^{\circ}$ C, employing mobile phase containing Acetonitrile: Double Distilled Water: Glacial Acetic Acid (75:25:0.2) delivered at the flow rate of 1.0 ml/min. The retention time of BAT was found to be 7.1 ± 0.2 min and of LFT 11 ± 0.5 min. The calibration curve was linear in the concentration range for BAT (50–250 μg/ml) and for LFT (300–1500 μg/ml) in accordance with the amount present in marketed formulation (ratio of 1:6).

2.5. Screening of components (solubility studies)

The solubility studies of BAT in different oils, surfactants and solubilizers were previously done in lab (Joshi et al., 2008). In case of LFT, 10 mg of LFT was taken in test tubes. The oil/surfactant/solubilizer was individually added in portions to LFT and mixed till it was completely solubilized. The amount of oils, surfactants and solubilizers required to dissolve the drug was determined.

2.6. Construction of pseudo-ternary phase diagrams

The pseudo-ternary phase diagrams were constructed by water titration method (Joshi et al., 2008). Briefly homogenous liquid mixtures of oil, surfactant and co-surfactant were titrated with water till the appearance of permanent turbidity, at room temperature. Capmul® MCM was used as oil phase; Tween® 80 was used as surfactant. Mixtures of oil and surfactant were prepared varied from 9:1 to 1:9 weighed in screw-cap glass tubes and were vortexed. Each mixture was then slowly titrated with aliquots of distilled water and stirred at room temperature to attain equilibrium. The mixtures were visually examined for transparency. After equilibrium was reached, the mixtures were further titrated with aliquots of distilled water until they exhibited turbidity. Clear and isotropic samples were deemed to be within the microemulsion (ME) region. Based on the ME region, required percentage of oil, surfactant was selected, and used for preparation of self-micro emulsifying drug delivery system (SMEDDS) containing BAT.

2.7. Measurement of mean globule size

The globule size and polydispersity index (PI) of the resultant ME were determined by photon correlation spectroscopy (PCS) on N4 Plus Submicron Particle Size Analyzer (Beckman Coulter, USA) at a scattering angle of 90°. Each unit dose of SMEDDS containing 20 mg of BAT was diluted with 900 ml of the double distilled water, filtered through 0.45 μm membrane filter (Pall Life Sciences, India). All measurements were performed in triplicate at a temperature of $25.0 \pm 2.0^{\circ}$ C.

2.8. Formulation development

In case of BAT, different compositions with higher proportion of oil (to achieve higher solubility of drug in system) were selected from the ME region to formulate the SMEDDS.

Download English Version:

https://daneshyari.com/en/article/2502114

Download Persian Version:

https://daneshyari.com/article/2502114

Daneshyari.com