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Enhanced brain delivery with lower hepatic exposure of lazaroid loaded nanostructured lipid carriers developed using a design of experiment approach☆

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ABSTRACT

The current study was designed to develop and optimize lazaroid loaded nano-structured lipid carriers (LAZ-NLCs) using design of experiment approach for enhancing lazaroid brain exposure. Response surface plots were used to determine the effects of independent variables (amount of PEGylating agent and liquid lipid) on dependent variables (particle size, zeta potential and encapsulation efficiency), while numerical optimization was used for optimizing LAZ-NLCs composition. The optimal LAZ-NLCs were spherical in shape with measured size of 172.3 ± 3.54 nm, surface charge of −4.54 ± 0.87 mV and encapsulation efficiency of 85.01 ± 2.60%. The optimal LAZ-NLCs were also evaluated for hemolytic potential, storage stability and solid-state properties. The plasma pharmacokinetics along with brain and hepatic distributions of control lazaroid citrate solution and optimal LAZ-NLCs formulation were evaluated in Sprague-Dawley rats after the single bolus intravenous administration. The optimized LAZ-NLCs and the control lazaroid citrate solution had similar plasma pharmacokinetic profiles; however, differential organ bio-distributions were observed. The lazaroid exposure in brain was enhanced by two times with a decreased liver exposure by half for the NLCs group compared to the solution group.

1. Introduction

The 21-aminosteroids or lazaroids are a group of compounds known to be potent inhibitors of lipid peroxidation (*in vitro* and *in vivo*), exerting its effect by radical scavenging action and preferential accumulation in vascular endothelium leading to membrane stabilization (Kavanagh and Kam, 2001). Lazaroid U-74389G (LAZ) has specifically shown to inhibit radiation induced lipid peroxidation, protect the normal brain tissues without protecting tumor tissues and has demonstrated anti-proliferative activity *in vitro* (Buatti et al., 1996; Durmaz et al., 1999; Kondziolka et al., 1999). Glioblastoma accounts for almost 80 percent of all malignant primary brain tumors (Dolecek et al., 2012). The current standard treatment involves maximal surgical removal of the tumor followed by concurrent radiation therapy and chemotherapy (anti-tumor drugs) with temozolomide, with median survival duration of approximately 14 months (Bartek et al., 2012). One of the major problems associated with the current glioblastoma therapy is the radiation induced lipid peroxidation (Gulbahar et al., 2009) and resistance to temozolomide (Sze et al., 2013). LAZ could potentially be used as an alternative agent for the dual purposes of radio-protection and tumor-suppression in the treatment of glioblastoma.

LAZ has high hepatic clearance on intravenous administration. Additionally, LAZ is metabolized via both oxidative (cytochrome P450) and reductive (5α-reductase) pathways (Wienkers et al., 1995), and are potential substrates of P-glycoprotein (P-gp) efflux transporter (Microfilms and International, 2008). These pharmacokinetic characteristics decrease the systemic exposure of LAZ and limit its transport across blood brain barrier (BBB). The solubility of LAZ is limited by pH with a maximum solubility of 2 mg/mL at pH 3.0 using buffered citrate solution. The LAZ citrate solution on intravenous use results in pain and irritation at the site of injection, and thus limits its bolus administration.

A formulation approach using nano-structured lipid carriers (NLCs), the second generation of solid lipid nanoparticles (SLNs) with higher drug payload and longer storage stability than SLNs, was explored to modify the pharmacokinetics and distribution of LAZ for maximizing its

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delivery to the brain. NLCs due to their sub-micron size and surface modifications can evade the uptake by the reticuloendothelial system (RES), resulting in prolonged systemic drug circulation and crossing the BBB for a better exposure to the brain. Additionally, NLCs have advantages over polymeric nanoparticles and liposomes (Joshi and Muller, 2009; Ye et al., 2016). NLC preparation involves mixing of spatially different liquid lipids or oils into the highly ordered state of solid lipids to yield imperfect, less ordered crystal lattice which is capable of increasing drug encapsulation and preventing drug expulsion during storage (Müller et al., 2002).

NLCs are predominantly used for topical administration and only limited studies are reported for exploring the brain exposure of drug loaded NLCs via intravenous route (Hsu et al., 2010; Jiang et al., 2015; Li et al., 2010; Lim et al., 2014; Tsai et al., 2012). Also, the use of design of experiment (DoE) approach in development of NLCs is limited to optimizing formulation components such as surfactant amount, drug to lipid ratio and process parameters such as ultrasonication or homogenization cycles (Gupta et al., 2015; Hejri et al., 2013; Zhang et al., 2013). The effects of surface modifiers like PEG and solid to liquid lipid ratios on physico-chemical properties of NLCs for enhancing brain exposure have not yet been systematically studied using DoE approach. Design of experiment (DoE) approach involves creating a design space for determining the relationship between factors affecting a process and the output of that process. Building an experimental design involves the statistical modeling to provide maximum information with a minimal number of experiments, and simultaneous multivariate analysis along with its interactions (Shivhare, 2010). Response surface methodology (RSM) is a type of DoE for developing, improving, and optimizing processes (Myers et al., 2016). Central composite design (CCD) involving the use of RSM is a factorial or fractional factorial design with center points and supplemented by a group of axial points (star points) for estimating response surface curvature.

We are reporting, for the first time, a systematically developed formulation of LAZ loaded NLCs, intended for intravenous administration to enhance LAZ brain exposure as a potential treatment in glioblastoma. The aim of this research was to utilize a logical and rational approach using CCD to determine the effects of formulation compositions on NLCs properties, and assist in selection of optimum NLCs composition. The optimal LAZ-NLCs were characterized for physico-chemical properties such as morphology (TEM), physical state and crystallinity (DSC and XRD), *in vitro* hemolytic potential and storage stability. Moreover, the plasma pharmacokinetics along with brain and hepatic biodistribution of LAZ with the optimal LAZ-NLCs formulation were evaluated in Sprague-Dawley rats after a single bolus intravenous administration, using LAZ solution as a reference.

2. Materials and methods

2.1. Materials

Lazaroid U74389G (LAZ) was purchased from Enzo Life Sciences Inc. (Farmingdale, NY, USA). Diadzein, used as internal standard (IS) was purchased from LC Laboratories (Woburn, MA, USA). Glyceryl behenate (Compritol 888 ATO), PEG-8 caprylic/capric glyceride (Labrasol), medium chain triglyceride (Labrafac™ Lipophile WL 1349) and glyceryl mono-oleate (Pecol) were purchased from Gattefosse (Saint-Priest Cedex, France). Trimyrsitin (Dynasan 114) and tristearin (Dynasan 118) were purchased from Cremer Oleo Division (Witten, Germany). Safflower and flaxseed oil were purchased from Jedwards International, Inc. (Braintree, MA, USA). Oleic acid was purchased from Sigma Aldrich (St. Louis, MO, USA). Soybean oil and polysorbate 80 were purchased from PCCA (Houston, TX, USA). mPEG-DSPE, MW: 2000 (DSPE-PEG 2 k) was purchased from Nanocs (Boston, MA, USA). Tetra-ethyl ammonium acetate was purchased from Acros Organics (NJ, USA). Amicon Ultra-4 centrifugal filters MWCO: 10,000 were purchased from Merck Millipore (Cork, Ireland). Male Sprague Dawley rats (250–300 g) with jugular vein cannula were purchased from Harlan (Indianapolis, IN, USA). All solvents used were of LC/MS grade and purchased from EMD Millipore (Billerica, MA, USA).

2.2. Selection of lipids

2.2.1. Selection of liquid lipid (oil)

The oil component of NLCs was selected by evaluating the saturation solubility of LAZ in different oils. An excess amount of LAZ was added to 0.5 mL of the oil and was shaken for 40 h at 37 °C. LAZ was stable (> 90%) at this experimental condition. The oil and LAZ mixtures were centrifuged at 17,968 × *g* for 20 min. The supernatant was diluted with 50:50 v/v mixtures of methanol and chloroform, and analyzed using the validated HPLC method described in Section 2.5.7.

2.2.2. Selection of solid lipid

There is no direct method to determine the solubility of LAZ in solid lipids. The main role of solid lipid component of NLCs is to ensure the maximum drug solubilization and sustained drug release over a period of time. The solubility of LAZ in various solid lipids trimyrsitin (TM), tristearin (TS) and glyceryl behenate (GB) was estimated semi-quantitatively by assessing the melting point transition of LAZ mixed with solid lipids in ratio of 1:5, using differential scanning calorimetric (DSC) analysis (Liu et al., 2014). The conditions for DSC analysis are described in Section 2.5.4. Furthermore, LAZ loaded SLNs were prepared using the three aforementioned lipids in a similar manner as in Section 2.3. The *in vitro* release profiles (N = 3, Mean ± SD) of the LAZ loaded SLNs incubated in plasma were measured using a modified protocol from Nornoo and Chow (2008). The solid lipid was selected based on the results of DSC analysis, and the low percentage of cumulative drug release from the prepared SLNs.

2.3. Preparation of LAZ-NLCs

LAZ-NLCs were prepared using a simple stirring and ultra-sonication method as previously described (Venkateswarlu and Manjunath, 2004) with some modifications (Fig. 1). The lipid phase, consisting of LAZ (20 mg), lecithin (100 mg), liquid lipid, solid lipid and DSPE-PEG 2 k, was dissolved in 2 mL mixture of methanol and chloroform (50:50 v/v). The organic solvents were removed completely under reduced pressure using Buchi Rotavapor R200 (Flawil, Switzerland). The LAZ containing lipid matrix was heated at 75 °C. The aqueous phase containing Polysorbate 80 (100 mg) dissolved in 10 mL of double distilled water was also heated at 75 °C and added drop-wise with stirring to the drug loaded lipid melt. The resulting coarse oil in water emulsion was ultra-sonicated using ultra-sonic processor Q500 (Qsonica, Newton, CT, USA) for 1 min at 30% amplitude to obtain hot nano-emulsion. The LAZ-NLCs were obtained by cooling the hot nano-emulsion in a water

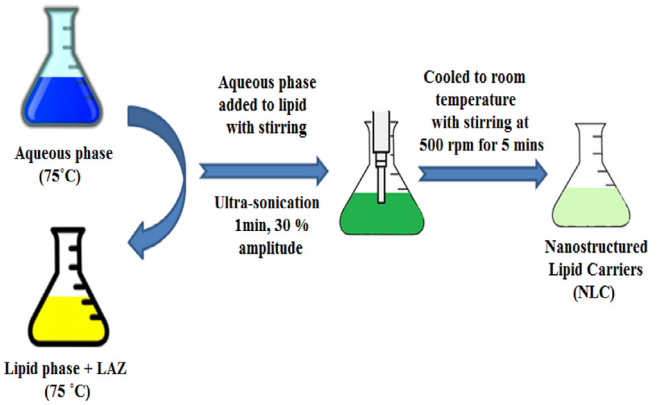

Fig. 1. Schematic outline for preparation of LAZ-NLCs using ultrasonication.