International Journal of Pharmaceutics 473 (2014) 442–457

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

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

Pharmaceutical nanotechnology

Effect of surface charge on the brain delivery of nanostructured lipid carriers *in situ* gels via the nasal route

Yasmine M. Gabal [a], Amany O. Kamel [a,c,*], Omaima A. Sammour [a], Ahmed H. Elshafeey [b,c]

[a] Pharmaceutics and Industrial Pharmacy Department, Faculty of Pharmacy, Ain Shams University, Cairo, Egypt
[b] Pharmaceutics and Industrial Pharmacy Department, Faculty of Pharmacy, Cairo University, Cairo, Egypt
[c] School of Pharmacy, University of Waterloo, 200 University Ave. W., Waterloo, ON N2L 3G1, Canada

ARTICLE INFO

Article history:
Received 10 February 2014
Received in revised form 4 July 2014
Accepted 20 July 2014
Available online 22 July 2014

Keywords:
Nanostructured lipid carriers
Surface charge
Brain delivery
Intranasal
In vivo

ABSTRACT

The aim of this study was to investigate the influence of the nanocarrier surface charge on brain delivery of a model hydrophilic drug via the nasal route. Anionic and cationic nanostructured lipid carriers (NLCs) were prepared and optimized for their particle size and zeta potential. The optimum particles were incorporated in poloxamer *in situ* gels and their *in vivo* behavior was studied in the plasma and brain after administration to rats. Optimum anionic and cationic NLCs of size <200 nm and absolute zeta potential value of ≈34 mV were obtained. Toxicity study revealed mild to moderate reversible inflammation of the nasal epithelium in rats treated with the anionic NLCs (A7), and destruction of the lining mucosal nasal epithelium in rats treated with the cationic NLCs (C7L). The absolute bioavailability of both drug loaded anionic and cationic NLCs *in situ* gels was enhanced compared to that of the intranasal solution (IN) of the drug with values of 44% and 77.3%, respectively. Cationic NLCs *in situ* gel showed a non significant higher C_{max} (maximum concentration) in the brain compared to the anionic NLCs *in situ* gel. Anionic NLCs *in situ* gel gave highest drug targeting efficiency in the brain (DTE%) with a value of 158.5 which is nearly 1.2 times that of the cationic NLCs *in situ* gel.

1. Introduction

Recently drug delivery to the brain has attracted great attention, however, the blood–brain barrier (BBB) acts as an obstacle to brain delivery of hydrophilic and high molecular weight drugs. The limitation of drug uptake by the brain is due to the lack of paracellular opening in the BBB, absence of pinocytosis as well as protein mediated efflux (Smith, 2003). Hence, with the emerging of new potential therapeutic agents which possess physicochemical properties that hinder their delivery to the brain, there is a great need for efficient delivery systems to enhance their brain uptake.

Colloidal nanocarriers have proved great potential in targeting drugs to the required tissue with successful intracellular retention. The effect of surface charge in enhancing the transport of nanoparticles across the BBB is controversial. Most endothelial cells and the surface glycocalyx layer of the BBB are anionic due to sialic acid residues of concentrated acidic glycoproteins (Ribeiro et al., 2012). Thus cationic nanoparticles are speculated to be easily attracted to the brain endothelial cells. This electrostatic attraction increases the contact time of cationic nanoparticles with the BBB and thus enhances their brain penetration via adsorptive mediated endocytosis (Drin et al., 2003). On the other hand, contrary to the hypothesis that anionic nanoparticles are repelled by the anionic charge at the BBB, previous literatures (Lockman et al., 2004; Tosi et al., 2008) indicated that both cationic and anionic nanoparticles can cross the BBB. Hence, both the particle size and the surface charge of nanocarriers affect the nanoparticles permeation into the brain.

NLCs are colloidal carriers that are used as targeted drug delivery systems due to their several advantages including controlled and targeted drug release, possible high drug payload, feasibility of carrying both hydrophilic and lipophilic drugs, avoidance of organic solvents being a water based formulation, biocompatibility, biodegradability with no reported biotoxicity of the lipid carrier system, improved drug stability, lower cost than polymeric or surfactant based carriers, easy to scale up, sterilize, validate and gain regulatory approval (Muller et al., 2002a).

Intranasal route is a potential route for drug delivery to the brain. Intranasally administered drugs enter the brain through three different pathways: systemically where the drug crosses the BBB, through the olfactory region and the trigeminal neural

* Corresponding author at: School of Pharmacy, University of Waterloo, 200 University Ave. W., Waterloo, ON N2L 3G1, Canada.
Tel.: +20 1 0011429211; fax: +20 2 24051106.
E-mail address: amany.kamel@yahoo.com (A.O. Kamel).

http://dx.doi.org/10.1016/j.ijpharm.2014.07.025
0378-5173/

pathway where it is transported directly from the nasal cavity to the central nervous system (Kumar et al., 2008).

The purpose of this work was to study the effect of nanocarrier surface charge on the *in vivo* brain delivery of a hydrophilic drug through the nasal route. Anionic and cationic NLCs encapsulating ropinirole hydrochloride (RP HCl) as a model drug were optimized for their particle size and zeta potential. Furthermore, their *in vivo* behavior was investigated in the plasma and brain after intranasal administration of their poloxamer *in situ* gels to rats.

2. Materials and methods

2.1. Materials

Ropinirole HCL (RP HCl) (Galaxosmithkline, USA) was kindly gifted by EVA Pharma (Cairo, Egypt). Compritol 888 ATO (28–32% glyceryl tribehenate, 52–54% dibehenate, and 12–18% monobehenate) and Labrafac lipophile WL1394 (triglyceride medium chain, caprylic/capric TG) were kindly gifted by Gattefosse (Saint-Priest, France). Stearylamine (SA), Poloxamer 407 (Pluronic F127) and Poloxamer 188 (Pluronic F68) were purchased from Sigma Chemical Co. (USA). Tween 80 was purchased from Merck Chemicals (Germany). Soya phosphatidylcholine 99% (Epikuron 200) was purchased from Degussa Texturant Systems (Deutschland, Hamburg, Germany). Deoxycholic acid sodium salt (SDC) was purchased from Fluka Co. (USA). HPMC (hydroxypropyl methyl cellulose) (Methocel E5) was gifted by Egyptian International Pharmaceutical Industries, EIPICO (Cairo, Egypt). Potassium dihydrogen phosphate and disodium hydrogen phosphate were purchased from Adwic, El-Nasr Pharmaceutical Co. (Cairo, Egypt). All chemicals are of analytical grade. Spectra/Por dialysis membrane (12,000–14,000 molecular weight cut off) was purchased from Spectrum Laboratories Inc. (Rancho Dominguez, CA, USA).

2.2. Preparation of NLCs

2.2.1. Anionic NLCs

Anionic RP HCl-loaded NLCs were prepared by hot high shear homogenization method (Mehnert and Mader, 2001). Briefly, Compritol 888 ATO and Labrafac™ Lipophile WL1349 (in the ratio 7:3) as the lipid phase were melted at 80 °C; the total amount of lipids was kept constant at 5 wt%. Then various concentrations of RP HCl (wt% of the lipid content) were dispersed in the molten lipids. At the same time, an aqueous surfactant solution was heated to the same temperature so that the total volume of preparation was 10 mL. Both the aqueous phase and the lipid were heated and stirred on magnetic stirrers. The hot surfactant solution was then dispersed in the hot lipid phase using a preheated Ultra-Turrax T25 (80 °C) (Ultra Turrax T25; IKA, Staufen, Germany) to the same temperature (80 °C) at 12,000 rpm for 3 min. The obtained pre-emulsion was subsequently homogenized at 80 °C at 24,000 rpm for 1.5 min. The hot oil in water (o/w) nanoemulsion was then cooled to room temperature for the lipid phase recrystallization and NLCs formation which were then kept refrigerated at 4 °C. A full factorial design was built up to optimize the anionic NLCs. The study design involved the investigation of the effect of three independent variables *viz.* drug concentration, surfactant type and surfactant concentration. The drug concentration was studied at three levels (1%, 2.5%, and 5% of total lipid concentration). The surfactant type was studied at two levels *viz.* P188:Tween 80:SDC (2:2:1) and PC:P188:Tween 80:SDC (1:2:2:1). The surfactant concentration was studied at 2.5 and 5% w/w levels. The dependent variables investigated were the particle size and zeta potential of RP HCl loaded anionic NLCs. A design of the full factorial experiment as well as the composition of different formulae was shown in Table 1.

2.2.2. Cationic NLCs

Cationic RP HCl-loaded NLCs were prepared adopting the same procedure used for preparing anionic NLCs with the addition of 0.3 wt% SA to be melted with the lipid phase. The study design involved the investigation of the effect of four independent variables, *viz.* drug concentration at three levels (1%, 2.5%, 5% of total lipid concentration), surfactant type at two levels (P188: Tween 80:SDC and PC:P188:Tween 80:SDC), surfactant concentration at two levels (2.5% and 5% w/w) and SDC concentration at two levels (high and low). SDC amount was decreased to a suitable concentration because being anionic in nature, it counteracts the positive charge induced by SA leading to lower zeta potential values and larger sized particles. Hence, according to our preliminary study, for 1% RP HCl, the surfactant to SDC ratios, P188:Tween 80:SDC (2:2:0.25) and (2:2:0.5) were used at low and high levels of SDC, respectively. However, cationic NLCs prepared with higher drug concentrations 2.5% and 5% required higher SDC concentration to neutralize RP HCl and improve its entrapment efficiency (EE%). Thus higher ratios of SDC to the used surfactants P188:Tween 80:SDC (2:2:0.5) and (2:2:1) were used at low and high levels of SDC, respectively. A typical design and composition of different formulae are shown in Table 2.

2.3. Characterization of NLCs

2.3.1. Transmission electron microscope (TEM)

The morphology of the cationic and anionic RP HCl loaded NLCs was examined by the transmission electron microscope (JEM-1010; JEOL Ltd. Tokyo, Japan). One drop of diluted NLCs

Table 1
Composition of the prepared RP HCl loaded anionic NLCs, and their measured responses.

Surfactant types/ratios	Drug concentration (%wt)	Surfactant concentration (%wt)	NLC code	Measured responses			
				Mean particle diameter (nm) ± SD	PDI ± SD	Zeta potential (mV) ± SD	EE% ± SD
P188:Tween 80:SDC (2:2:1)	1	2.5	A1	222.8 ± 1.5	0.45 ± 0.04	−37 ± 2	73.7 ± 0.4
		5	A2	182.7 ± 7.5	0.39 ± 0	−36 ± 2.3	56.7 ± 3
	2.5	2.5	A3	273 ± 7.6	0.46 ± 0.04	−39 ± 2.5	54.2 ± 0.2
		5	A4	199 ± 14.1	0.41 ± 0.03	−38 ± 1.3	55.1 ± 4.5
	5	2.5	A5	250 ± 1.6	0.44 ± 0.01	−37 ± 4.3	42.2 ± 1.2
		5	A6	211 ± 12.4	0.43 ± 0.06	−39 ± 2	46.6 ± 2.3
PC:P188:Tween 80:SDC (1:2:2:1)	1	2.5	A7	175 ± 0.9	0.26 ± 0.01	−34 ± 2.1	52.8 ± 0.7
		5	A8	113 ± 1	0.30 ± 0.02	−23 ± 1.2	54.3 ± 3.6
	2.5	2.5	A9	177 ± 6.3	0.32 ± 0.07	−31 ± 0.5	44.9 ± 0.4
		5	A10	153 ± 36.8	0.33 ± 0.07	−33 ± 1.8	56.7 ± 4.7
	5	2.5	A11	216 ± 8.3	0.34 ± 0.03	−32 ± 2.8	35.5 ± 3.8
		5	A12	103 ± 1	0.33 ± 0.03	−26 ± 6.6	42.1 ± 8.9