European Journal of Pharmaceutical Sciences 114 (2018) 103–113

Contents lists available at ScienceDirect

European Journal of Pharmaceutical Sciences

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

Mannose-functionalized solid lipid nanoparticles are effective in targeting alveolar macrophages

Ana Costa[a,b,c], Bruno Sarmento[a,b,d,*], Vítor Seabra[d]

[a] i3S, Instituto de Investigação e Inovação em Saúde, Rua Alfredo Allen 208, 4200-135 Porto, Portugal
[b] INEB, Instituto de Engenharia Biomédica, Rua Alfredo Allen 208, 4200-135 Porto, Portugal
[c] ICBAS, Instituto Ciências Biomédicas Abel Salazar, Rua Jorge Viterbo Ferreira 228, 4050-223 Porto, Portugal
[d] CESPU, Instituto de Investigação e Formação Avançada em Ciências e Tecnologias da Saúde, Rua Central de Gandra 1317, 4585-116 Gandra, Portugal

ARTICLE INFO

Keywords:
Solid lipid nanoparticles
Mannose functionalization
Alveolar macrophage
Macrophage nanoparticle uptake

ABSTRACT

Mannose receptor is highly expressed on alveolar macrophages, being a potential target to promote the specific local drug delivery of anti-tuberculosis agents through the use of functionalized nanocarriers. In this work, isoniazid (Isn)-loaded solid lipid nanoparticles (SLN), reinforced with stearylamine (SA) were produced by double emulsion technique and further surface-functionalized with mannose in a straightforward chemical approach. Upon pre-formulation assessment, SLN close to 500 nm average size, positively charged and with association efficiency of ISN close to 50% were obtained. Functionalization with mannose was performed after SLN production and confirmed by Fourier transform infrared spectroscopy (FTIR). Both functionalized and non-functionalized SLN demonstrated to devoid of toxicity when tested in human lung epithelial cell line (NCI-H441) and differentiated THP-1 (dTHP-1), reducing the intrinsic cytotoxicity of Isn when incorporated into SLN. Uptake studies were conducted on same macrophage-like cells and the results showed that fluorescent mannosylated SLN (M-SLN) were more efficient in be internalized comparatively to SLN. Moreover, the uptake of M-SLN was reduced when cells were pre-incubated with mannose, demonstrating the receptor-dependence internalization of functionalized SLN. These functionalized nanocarriers may represent a useful platform to target alveolar macrophages for delivering anti-infective drugs.

1. Introduction

The surface of alveolar epithelium is a crescent relevant gateway for drug delivery, being mainly covered by squamous alveolar type I cell that delimitates the alveolar space from the endothelium layer and by a few percentage of alveolar type II cell that produce surfactant, cytokines and chemokines for the maintenance of lung homeostasis and also for the epithelium regeneration upon injury (Chuquimia et al., 2013). On the surface of this physiologic barrier there are important resident cells, namely alveolar macrophages that present the ability to phagocyte airborne particles and pathogenic agents at alveolar space (Gordon and Read, 2002; Miyata and van Eeden, 2011). Those cells also have an impact on lung homeostasis, since they present the ability to produce anti-inflammatory cytokines (IL-10) that avoids the inflammatory response when exposed to harmless antigens (Chanteux et al., 2007), or can mediate an opposite reaction by promoting the release of inflammatory mediators and reactive oxygen species, as well the neutrophils recruitment towards alveolar space. Moreover, during inflammatory process the phagocytic capacity is enhanced as well as the ability to process and present antigens on its surface (Forbes et al., 2014).

The presence of alveolar macrophages at alveolar space represents a duality regarding the pulmonary administration of nanocarriers. Due to its phagocytic ability, they are able to clear the nanocarriers from alveolar surface, originating a reduction of local and systemic drug bioavailability (Amoozgar and Yeo, 2012). Consequently, different surface modification on nanocarriers has been made to promote stealth properties, namely by coating with polyethylene glycol (Amoozgar and Yeo, 2012), peptides (Rodriguez et al., 2013) or chitosan (Trapani et al., 2013). However, the phagocytic properties of these cells can be advantageous when a system should be targeted towards the alveolar macrophages, especially for treatment intracellular bacterial infections, as tuberculosis. *Mycobacterium tuberculosis*, the agent responsible for this lethal disease, infects the alveolar macrophages and survive inside macrophages on a latent state (Gupta et al., 2012). Due to toxic effects and inefficacy of the conventional anti-tuberculosis drug on the market, different nanocarriers has been developed for the treatment of *Mycobacterium tuberculosis* (Clemens et al., 2012; Gaspar et al., 2016; Kumar

* Corresponding author at: INEB, Instituto de Engenharia Biomédica, Rua Alfredo Allen 208, 4200-135 Porto, Portugal.
E-mail address: bruno.sarmento@ineb.up.pt (B. Sarmento).

https://doi.org/10.1016/j.ejps.2017.12.006
Received 29 October 2017; Received in revised form 3 December 2017; Accepted 7 December 2017
Available online 08 December 2017
0928-0987/

et al., 2006; Maretti et al., 2014).

In order to enhance the internalization of nanocarrier by alveolar macrophages and consequently to improve the drug accumulation on lung tissue, surface modification of nanocarriers with different ligands can also be performed (Costa et al., 2015). Mannose is one of the most common ligands used to decorate the surface of these nanosystems, once the alveolar macrophages express mannose receptors (CD206) on its surface. This receptor has eight C-type carbohydrate-recognition domains (CRDs) with the ability to recognize D-mannose, *N*-acetylglucosamine and L-fucose residues presented on the surface of pathogens and also endogenous ligands, namely lutropin and thyrotropin (Garcia-Vallejo and van Kooyk, 2009). Mannose receptor is involved on recognition of pathogens, on antigen processing and presentation (Staines et al., 2014; Vigerust et al., 2012). Lastly it presents an important role on clearance of *Mycobacterium tuberculosis* (Irache et al., 2008): the CDR of mannose receptor is able to recognize the mannose-capped lipoarabinomannan (ManLAM), a component of bacteria wall that causes the phagocytosis of bacilli by macrophages, and the inhibition phagosome–lysosome fusion, enabling the bacteria survival inside of macrophage (Kang et al., 2005). Therefore, the preparation of mannosylated formulation may be a good approach for targeting drugs towards alveolar macrophages. Besides the ligand-anchored to nanocarrier, the mean particle size, surface, morphology or even the inherent composition of nanosystem may influence the internalization of the nanocarriers by macrophages (Hirota et al., 2007; Makino et al., 2003; Park et al., 2013).

In this study different SLN were optimized by screening the lipid matrix, the inner phase composition, the surfactant phase, and last, the amount of SA. The final formulation with best physical-chemical properties that might promote its internalization by macrophages was further functionalized with mannose according to the methodology described before (Jain et al., 2010; Kumar et al., 2006). As a model drug it was selected Isn, a hydrophilic drug used for *Mycobacterium tuberculosis* treatment.

2. Materials and methods

2.1. Materials

Witepsol® E85 (hydrogenated coco-glycerides, m.p. 42–44 °C) and Compritol® 888 ATO(glyceryl behenate, m.p. 70 °C) were kindly supplied by Sasol (Witten, Germany) and Gattefossé (Saint Priest, France), respectively; Stearic acid (Edenor ST1 GW) come from Oleo Solutions (York, United Kingdom); Isn, paraformaldehyde (PFA), ethyl acetate and sodium acetate were bought from Merck (Darmstadt, Germany); Tween® 80, poly(vinyl alcohol) (PVA) (87–90% hydrolyzed, average molecular weight 30,000–70,000), coumarin-6, stearylamine (SA) 97%, phosphate buffered saline (PBS), D-(+)-mannose, phorbol 12-myristate 13-acetate (PMA), trimethylamine HPLC gradient grade and dichloromethane (DCM) (99.9% purity), Manucol LD and Keltone LV were purchased from Sigma-Aldrich (St. Louis, USA); Methanol and acetonitrile (ACN) HPLC gradient grade were from Fisher Scientific (Leicestershire, UK); Trifluoroacetic acid (TFA) was purchased from Acros Organic (New Jersey, USA); 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT), 4′,6-Diamidino-2-Phenylindole, Dihydrochloride (DAPI), dimethyl sulfoxide (DMSO), sodium pyruvate and trypsin were from Sigma (St. Louis, USA); RPMI-1640, hank's balanced salt solution (HBSS), versene were purchased from Gibco (Waltham, USA); Fetal Bovine Serum (FBS) was from Biochrom (Cambridge, UK); Penicillin-streptomycin (Pen/strep) was from Biowest (Nuaillé, France); Lactate dehydrogenase (LDH) kit was purchased from Takara Bio Inc. (Shiga, Japan); Triton X-100 was purchased from Spi-Chem (Atlanta, USA); CellMask™ Deep Red Plasma membrane Stain was purchase from Life technologies (Carlsbad, USA); Interleukin 4 (IL-4) was purchase from ImmunoTools (Friesoythe, Germany). Milli-Q® water and PBS for cell culture were produced in-house.

2.2. Preparation and characterization of Isn-loaded SLN

2.2.1. Preparation of Isn-loaded SLN

To produce Isn-loaded SLN, three different solid lipids commonly used for pulmonary drug delivery were selected and tested, namely Witepsol® E85, stearic acid and Compritol® 888 ATO (Weber et al., 2014). SLN were produced through a modified solvent emulsification-evaporation method based on a water/oil/water (W/O/W) double emulsion technique (Sarmento et al., 2007; Soares et al., 2013). Briefly, the organic phase was composed by 200 mg of lipid and 2 mg of SA dissolved in 2 mL of DCM, while the inner phase was constituted by 200 µL of Isn aqueous solution containing 5, 10 or 20 mg of Isn. After adding the aqueous solution to the organic solution, the mixture was sonicated through a sonication probe (Vibra-Cell™ultrasonic processor, Sonics & Materials, Inc., Newtown, USA), during 30 s with an amplitude of 70%; the formed W/O primary emulsion was dispersed in 8 mL of surfactant solution (PVA 1% (w/v)) and sonicated for another 30 s. The W/O/W double emulsion formed was poured into 15 mL of PVA solution and left under magnetic stirring during 3 h for organic solvent evaporation.

Based on physical-chemical properties of SLN of formulation, Witepsol® E85 was selected as the main component of lipid matrix, further modification on Isn-loaded SLN were performed, through the modification of inner phase, organic solvent and the surfactant solution. The lipid matrix (constituted by 200 mg of Witepsol® E85 and 2 mg of SA) and the amount of drug (10 mg of Isn) at inner phase were kept in all formulation. The parameters changed are described on the following Table 1, and all formulations were prepared according to the methodology described above in this section. The z-average, polydispersity

Table 1
Different modifications of Witepsol® E85-based SLN and respective physical-chemical characterization.

Inner aqueous phase	Organic phase	Surfactant (w/v)	Z-average (nm)	Pdi	Zeta potential (mV)	Association efficiency (%)
Milli-Q® water	DCM	PVA 1%	604.3 ± 29.4	0.43 ± 0.05	19.6 ± 3.5	24.2 ± 9.8
Milli-Q® water	DCM	PVA 2%	488.2 ± 6.2	0.36 ± 0.04	23.1 ± 2.2	13.6 ± 1.7
Milli-Q® water	DCM	Tween® 80 1%	511.8 ± 51.0	0.54 ± 0.03	15.8 ± 0.5	41.6 ± 1.8**
Milli-Q® water	Ethyl acetate	PVA 1%	192.1 ± 6.7*	0.12 ± 0.10**	31.8 ± 1.5***	7.7 ± 2.8*
PBS (pH 7.4)	DCM	PVA 1%	489.8 ± 7.8	0.41 ± 0.02	26.9 ± 2.2	50.2 ± 0.5**
PVA 1% (w/v)	DCM	PVA 1%	915.2 ± 74.7	0.48 ± 0.07	12.7 ± 2.3	15.3 ± 2.23
Manucol LD 1% (w/v)	DCM	PVA 1%	1398.7 ± 169.8***	0.94 ± 0.10***	9.8 ± 5.4**	NA
Keltone LV 1% (w/v)	DCM	PVA 1%	1509.0 ± 372.3***	0.91 ± 0.16***	7.6 ± 3.4***	NA

Mean ± S.D.; $n = 3$; NA: not assessed; The lipid matrix of each SLN contained 200 mg of Witepsol® E85 and 2 mg SA, while the inner aqueous phase had 10 mg of Isn. Z-average, Pdi, zeta potential and association efficiency of each modified SLN were compared with the characteristics of control (first row). Statistical analysis was performed through One-way ANOVA, with Dunnett's multiple comparison test.

* $p < 0.05$.

** $p < 0.01$.

*** $p < 0.001$.