Journal of Drug Delivery Science and Technology 43 (2018) 65–72

Contents lists available at ScienceDirect

Journal of Drug Delivery Science and Technology

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

Lipid and PLGA hybrid microparticles as carriers for protein delivery

Chengyu Wu, Stefania Baldursdottir, Mingshi Yang, Huiling Mu*

Department of Pharmacy, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark

ARTICLE INFO

Article history:
Received 28 July 2017
Received in revised form
29 August 2017
Accepted 6 September 2017
Available online 9 September 2017

Keywords:
Lipid excipients
PLGA
Hybrid microparticles
Protein release
Surface morphology

ABSTRACT

The present study aimed at investigating the influence of lipid excipients on protein carriers when proteins are encapsulated in lipid and PLGA hybrid particles. PLGA and lipid hybrid microparticles (MP) were prepared by a double emulsion method, and lysozyme was used as the model protein. The encapsulation efficiency (EE) of lysozyme in hybrid MP, particle surface morphology, as well as the release profile of lysozyme were investigated. The results showed that higher content of PLGA in the hybrid MP resulted in better EE of protein and smoother surface of the MP. Burst release of lysozyme from the MP was positively correlated to the chain length of acyl groups in lipids as well as the content of lipids in the hybrid MP. The polymorphic form of lipids in the hybrid MP affected both the EE of protein in the MP and the protein release from the MP, suggesting that EE of protein in the hybrid MP and the protein release profile could be regulated by changing lipid excipients as well as the level of lipids in hybrid MP. The present study provides a good basis for further investigation of the application potentials of lipid and PLGA hybrid MP in drug delivery.

1. Introduction

Therapeutic peptide and protein may provide special treatment on numerous diseases and disorders. Most of them have a short half-life and need to be injected frequently, therefore, formulations for long-term release of peptide and protein have gained great attentions [1,2]. There are a few PLGA microparticles (MP) products on the market for sustained delivery of peptides, such as leuprolide (Lupron Depot®, TAP Pharmaceutical Products Inc.), octreotide (Sandostatin LAR®, Novartis) and triptorelin (Trelstar®, Debiopharm). However, PLGA MP for sustained delivery of proteins is challenging and is still under development. One of the problems is the degradation of proteins during the preparation and storage of PLGA MP [3,4], especially the decreased pH during PLGA degradation affected the protein stability [5]. Additionally, non-specific adsorption to PLGA polymer may lead to incomplete protein release [6].

Lipids, one of the major human nutrients, are biocompatible and biodegradable. The safety and diversity of lipids have attracted more interests to their applications in drug delivery. It has been reported that triglyceride implant did not result in significant inflammatory response and cytotoxic reaction in mice over 2 months [7]. Additionally, the slow digestion rate of selected lipids might be applied to sustained delivery of peptide and protein drugs [8,9], for instance lipid MP have been used for sustained delivery of GnRH antagonist (antide) for 30 days *in vivo* [10].

Lipid and polymer hybrid system draw more and more attention in drug delivery system to merge benefit of lipid and polymer [11]. Our hypothesis was that adding lipids into PLGA MP could maintain the ability of MP for sustained drug release and avoid some of the disadvantages of PLGA MP. Limited studies have been conducted on the combination of lipids and PLGA in drug delivery in general, especially for sustained delivery of proteins. It has been reported that combing phospholipids with polymer affected drug release from the nanoparticles, which could result in delayed release or burst release of drug depending on the properties of phospholipids [12–14]. The phospholipids were mainly detected on the surface of polymer particles because of the amphiphilic property of phospholipid [15]. It has also been reported that addition of medium-chain triglyceride, tricaprin, in PLGA MP could accelerate drug release from the MP [16]. The present study aimed at achieving a better understanding of the effects of lipid structures and compositions on the release of proteins from PLGA MP. Therefore, lipid and PLGA hybrid MP were prepared with different contents of lipids in hybrid MP, as well as different lipid excipients, i.e. triglycerides containing medium-chain (TG8, TG12), long-chain (TG16) and very-long-chain fatty acids (TG22), and monoglycerides such as glycerol monostearate (GMS).

* Corresponding author. Department of Pharmacy, Universitetsparken 2, 2100, Copenhagen, Denmark.
E-mail address: huiling.mu@sund.ku.dk (H. Mu).

https://doi.org/10.1016/j.jddst.2017.09.006
1773-2247/

2. Materials and methods

2.1. Materials

The mono-acid triglycerides trilaurin (Dynasan D112, TG12), tripalmitin (Dynasan D116, TG16), and Tribehenate (Dynasan D122, TG22) were kindly donated by IOI Oleo GmbH (Hamburg, Germany). Captex 300 (medium chain triglycerides (MCT), caprylic (≈70%) and capric acids) was bought from ABITEC Corporation (Columbus, USA) and Geleol mono- and diglycerides NF (Glycerol monostearate 40–55 type 1, GMS) were obtained from Gattefossé (Lyon, France). PLGA (75/25, RG 753 S, inherent viscosity: 0.32–0.44 dl/g, around 50 KDa) was bought from EVONIK (Darmstat, Germany). Lysozyme from chicken egg (70.000 U/mg), sodium azide, polyvinyl alcohol (PVA, average MW 85.000–124.000, 87–89% hydrolyzed) were bought from Sigma Aldrich (St. Louis, MO, USA). All other regents were of analytical grade.

2.2. Microparticles preparation

Protein-loaded MP were prepared by double emulsion and solvent evaporation method based on the method described previously [17]. Briefly, 400 mg lipids and PLGA at different percentage were dissolved in 1 ml of chloroform (Table 1), mixed with 0.1 ml of lysozyme (50 mg/ml) and followed by probe sonication (Sonics & Materials, CT, USA). The probe sonication (130 W, 20 KHz) was operated at a pulse mode with 3 s work, and 3 s off, 60% of intensity, 10 times. Then, 3 ml of 2.5% PVA solution (room temperature) was added into the primary emulsion and probe sonicated again. The working condition of probe sonication was: 3 s work, 3 s off, 40% of intensity, 10 times. The obtained w/o/w emulsion was transferred into 200 ml of PVA solution (0.1%) at room temperature, which was stirred (200 rpm) for 4 h to evaporate the organic solvent. MP were obtained after centrifugation (6200 g, 10 min), and washed with purified water, followed by freeze-drying (0.08 Mbar, −50 °C, overnight). Table 1 lists the used lipid excipients (MCT, TG12, TG16, TG22, GMS), and the weight percentage of lipids in the formulations (5%, 10%, 20%, 33.3%, 50%, 66.7%).

2.3. Encapsulation efficiency determination

Lysozyme was extracted from the formulations using the method described previously with minor modification [18]. 10 mg of MP, accurately weighted, was dissolved in 10 ml of ethyl acetate at 40 °C, and lysozyme was pelleted by centrifugation at 1500 gfor 10 min. After removing the supernatant, the residual ethyl acetate was evaporated under nitrogen at room temperature. 2 ml of purified water was added to dissolve lysozyme, and the concentration of lysozyme was quantified by the HPLC method described below. The EE of lysozyme in the MP was calculated by:

EE% = Actual drug content in MP/Theoretical drug content*100

The HPLC method was slightly modified from the one reported by Christophersen et al. [19]. A Dionex HPLC system (Agilent Technologies, Waldbronn, Germany) was used with a C18 column (4.60 × 100 mm, 5 μm, 300 Å, Waters, USA). A binary solvent system was used at room temperature at a flow rate of 1 ml/min. Solvent A was 0.1% (v/v) trifluoroacetic acid (TFA) in purified water and solvent B was 0.1% (v/v) TFA in acetonitrile. A gradient method was used: 0–2 min: 23% B, 2–12 min: 23–50% B, 12–13 min: 50–100% B, 13–15 min: 100% B, 15–16 min: 100-23% B, 16–18 min: 23%. Lysozyme was detected at 220 nm by UV detection.

Table 1
Characterization of the various lipid PLGA hybrid MP.

Formulation	Lipids content (%)	Size (μm)	EE (%)
PLGA MP	0	14.69 ± 1.49	60.48 ± 1.84
TG16 PLGA MP	1.0	16.46 ± 2.37	55.48 ± 3.26
MCT PLGA MP	5.0	15.32 ± 2.00	60.29 ± 1.51
TG12 PLGA MP	5.0	24.16 ± 2.78*	62.67 ± 8.09
TG16 PLGA MP	5.0	13.04 ± 2.74	57.86 ± 4.45
TG22 PLGA MP	5.0	16.71 ± 1.03	47.38 ± 4.55*
TG16 PLGA MP	10.0	17.05 ± 1.89	50.19 ± 4.50
TG16 PLGA MP	20.0	16.76 ± 1.40	52.85 ± 13.64
TG16 PLGA MP	33.3	16.84 ± 1.16	42.61 ± 8.15*
TG16 PLGA MP	50.0	20.54 ± 2.60*	24.58 ± 6.10*
TG16 PLGA MP	66.7	17.31 ± 2.40	12.61 ± 6.57*
GMS PLGA MP	1.0	16.21 ± 4.16	54.70 ± 10.15
GMS PLGA MP	5.0	12.14 ± 4.17	17.33 ± 2.55*
GMS PLGA MP	10.0	12.17 ± 4.07	12.92 ± 5.44*

*: P < 0.05 vs PLGA MP, PLGA: poly (D, L-lactide-*co*-glycolide), EE: entrapment efficiency; TG12: triglycerides trilaurin; TG16: tripalmitin; TG22: Tribehenate; GMS: Glycerol monostearat; MCT: medium chain triglycerides, dominated by TG8:0. Results are given as average values ± S.D., n = 3.

2.4. Particle size analysis

The size and size distribution of MP were analyzed based on laser diffraction principle using Malvern Mastersizer 2000 (Malvern Instruments Ltd., UK) equipped with Hydro 2000S (Malvern Instruments Ltd., UK) for wet dispersions. The volume-mean diameter of MP was measured. Wet dispersions for particle size analysis were prepared as follows: MP suspension was transferred to the optical measurement cell containing the dispersing medium, i.e. 0.1% PVA solution. Measurements were performed while the sample was in the cell under stirring (1200 rpm) and ultrasound (50%). The obscuration was set between 8% and 12%.

2.5. Scanning electron microscopy (SEM)

Surface morphology of MP was observed by scanning electron microscopy (SEM) (Hitachi, TM3030, Japan) after particle preparation. The samples were coated with gold under an argon atmosphere for 20 s and examined under an accelerating voltage of 5 kV.

2.6. Differential scanning calorimetry (DSC)

DSC (Perkin Elmer, Shelton, USA) measurements were carried out to determine the polymorphic changes of lipids and glass transition points of PLGA. Approximately 5 mg of samples were weighed into DSC pans and crimp sealed. They were heated from 10 °C to 100 °C at a heating rate of 10 °C per min under a 20 ml/min of nitrogen. DSC system was controlled by the software Trios v3.3.1.

2.7. X-ray powder diffraction (XRPD)

X-ray diffraction measurements were performed to elucidate the solid state of TG16 and PLGA in hybrid MP both after particle preparation and *in vitro* release experiments by using a PANalytical X'Pert Pro diffractometer equipped with a PIXcel detector (PANalaytical B.V., Almelo, Netherlands). Measurements were conducted at ambient conditions by using Cu Kα radiation at 40 mA and 45 kV, with an angular increment of 0.04°/s and count time of 2 s. Data were collected at 0.05° (2 theta) within a 5°–35° range. Data were analyzed by X'Pert high score plus version 2.2.4 (PANalaytical B.V.).

2.8. In vitro release profiles

MP (approximately 50 mg, accurately weighed, $n = 3$) were suspended in 1.0 ml of the release medium (PBS-buffer pH 2.5,