International Journal of Pharmaceutics 493 (2015) 161–171

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

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

Pharmaceutical nanotechnology

Delivery of retinoic acid to LNCap human prostate cancer cells using solid lipid nanoparticles

Mushfiq H. Akanda[a], Rajeev Rai[a], Ian J. Slipper[a], Babur Z. Chowdhry[a], Dimitrios Lamprou[b], Giulia Getti[a], Dennis Douroumis[a,*]

[a] Faculty of Engineering and Science, University of Greenwich, Medway Campus, Chatham Maritime, Kent ME4 4TB, UK
[b] Strathclyde Institute of Pharmacy and Biomedical Sciences, University of Strathclyde, The John Arbuthnott Building, Glasgow G40NR, Scotland, UK

ARTICLE INFO

Article history:
Received 25 May 2015
Received in revised form 14 July 2015
Accepted 15 July 2015
Available online 19 July 2015

Key words:
Retinoic acid
Solid lipid nanoparticles
Cytotoxicity
Prostate cancer
Flow cytometry

ABSTRACT

In this study retinoic acid (RTA) loaded solid lipid nanoparticles (SLNs) were optimized by tuning the process parameters (pressure/temperature) and using different lipids to develop nanodispersions with enhanced anticancer activity. The RTA-SLN dispersions were produced by high-pressure homogenization and characterized in terms of particle size, zeta potential, drug entrapment efficiency, stability, transmission electron microscopy (TEM), atomic force microscopy (AFM), X-ray diffraction (XRD) and in vitro drug release. Thermal and X-ray analysis showed the RTA to be in the amorphous state, whilst microscopic images revealed a spherical shape and uniform particle size distribution of the nanoparticles. Anticancer efficiency was evaluated by incubating RTA-SLNs with human prostate cancer (LNCap) cells, which demonstrated reduced cell viability with increased drug concentrations (9.53% at 200 ug/ml) while blank SLNs displayed negligible cytotoxicity. The cellular uptake of SLN showed localization within the cytoplasm of cells and flow cytometry analysis indicated an increase in the fraction of cells expressing early apoptotic markers, suggesting that the RTA loaded SLNs are able to induce apoptosis in LNCap cells. The RTA-SLN dispersions have the potential to be used for prostate anticancer treatment.

1. Introduction

Solid lipid nanoparticles (SLNs) have attracted increasing attention as a promising colloidal carrier system, especially for lipophilic drugs (Chen et al., 2001; Mehnert and Mäder, 2001; Müller and Keck, 2004; Castelli et al., 2005). SLNs are spheres or platelets in the submicron size range (mainly between 150 and 300 nm). These are made of lipids, which are solid at room and body temperature and dispersed in an aqueous medium (Müller et al., 2011; Battaglia and Gallarate., 2012). SLNs are composed of a high melting point lipid as a solid core, which is coated by surfactants. Therefore, lipophilic drugs can be efficiently incorporated in the lipid core of SLNs. SLNs consist of a solid core rather than a fluid core, such as liposomes and emulsions, which facilitates prolonged and controlled release of drugs (Mehnert and Mäder, 2001). SLN incorporated drugs are protected against chemical degradation (Lim and Kim, 2002). The production of SLNs is easier to scale up compared with other drug delivery systems such as polymeric nanoparticles or liposomes (Mueller et al., 2000). The advantages of SLNs have driven numerous studies with various applications, particularly for parenteral administration of drugs. Typical parenteral SLNs applications include intra-articular to intravenous peroral administration (Muller et al., 2000) increased drug solubility (Yang et al., 2014) and pulmonary delivery (Yang et al., 2012). Studies performed by Gasco (1993) revealed that intravenously administered SLNs display prolonged drug plasma levels. Moreover, a much lower uptake by liver and spleen macrophages occurs due to the low surface hydrophobicity of SLNs avoiding the absorption of any blood proteins (Gasco, 1993; Göppert and Müller, 2005). Modified SLNs with an active targeting mechanism have been proved a promising drug delivery system with high specificity (Buhner, 2012,Gupta et al., 2007, Kakkar et al., 2013)

Retinoic acid (RTA) is a promising anticancer agent, which has been investigated in chemoprevention and treatment of cancer (Orlandi et al., 2002; Carneiro et al., 2012). The anticancer properties of RTA are achieved by binding to retinoic acid receptors or retinoid X receptors present on the nuclear membrane of cancer cells, leading to the induction of growth inhibition, differentiation

* Corresponding author at: Department of Pharmaceutical, Chemical & Environmental Sciences, Faculty of Engineering and Science, University of Greenwich, Medway Campus, Chatham Maritime, Kent ME4 4TB, UK. Fax: +44 0 208 331 9805.
E-mail address: D.Douroumis@gre.ac.uk (D. Douroumis).

http://dx.doi.org/10.1016/j.ijpharm.2015.07.042
0378-5173/

or apoptosis in these cells (Fang et al., 2002; Maden, 2007). RTA has already been used in clinical trials where RTA was given to cancer patients by oral administration, though the RTA concentration in blood circulation gradually decreases after long-term oral treatment. This phenomenon might occur due to the induced cytochrome P-450-dependent metabolism of RTA (Muindi et al., 1992). The poor aqueous solubility of RTA can be a major drawback for its parenteral administration. However, the incorporation of RTA in lipid-based carriers such as SLNs could be an attractive means to overcome such solubility limitations. The aim of this study was to develop stable RTA-SLN formulations with improved cellular uptake and anticancer efficacy in prostate LNCaP cancer cells.

2. Materials and methods

2.1. Materials

Retinoic acid was purchased from TCI (UK). Precirol® ATO 5 (PR) was purchased from Gattefosse. Poloxamer 188 (P188) was kindly donated by BASF (Ludwigshafen, Germany). LNCap cell line was purchased from the American Type Culture Collection (ATTC, Manassa, Virginia, USA). Stearic acid (SA), tristearin (TS), trilaurin (TL), Dulbecco's modified Eagle's medium (DMEM), thiazolyl blue tetrazolium bromide (MTT), L-glutamine, penicillin, streptomycin, heat inactivated fetal bovin serum (FBS) and trypsin were purchased from Sigma–Aldrich (UK). Rhodamine was purchased from Avanti polar lipids (USA). PE Annexin V Apoptosis Detection Kit I containing 7AAD as vital stain was obtained from BD Biosciences, UK. All the other chemicals and solvents used were of analytical or high-performance liquid chromatography (HPLC) grade.

2.2. Preparation of SLNs

SLNs were prepared by high pressure homogenization. In brief, appropriate amounts of lipids e.g., SA and P188 were accurately weighed and heated above the melting point of the lipid. For RTA loaded SLNs the drug was separately dissolved in ethanol (3 ml) and then added to the molten lipid. The drug containing the melted lipid was dispersed in a hot aqueous phase and homogenized with an UltraTurrax T25 (IKA®- WERKE GMBH, Staufen, Germany) homogenizer to form a pre-emulsion. The coarse dispersion produced was then transferred and homogenized in a Micro DeBee (South Easton, MA, USA) high pressure homogenizer at 15,000 psi for 7 min at 70 °C. The hot nano-dispersions were left to cool and allow the lipid to crystallize by forming lipid nanoparticles with a solid matrix.

2.3. Particle size analysis and zeta potential

The particle size distribution and zeta potential of the produced preparations were determined by dynamic light scattering photon correlation spectroscopy (PCS) using a Malvern Zetasizer Nano-ZS (Malvern, UK). The dispersions were adequately diluted with distilled water and measured in triplicate. The z-average and the

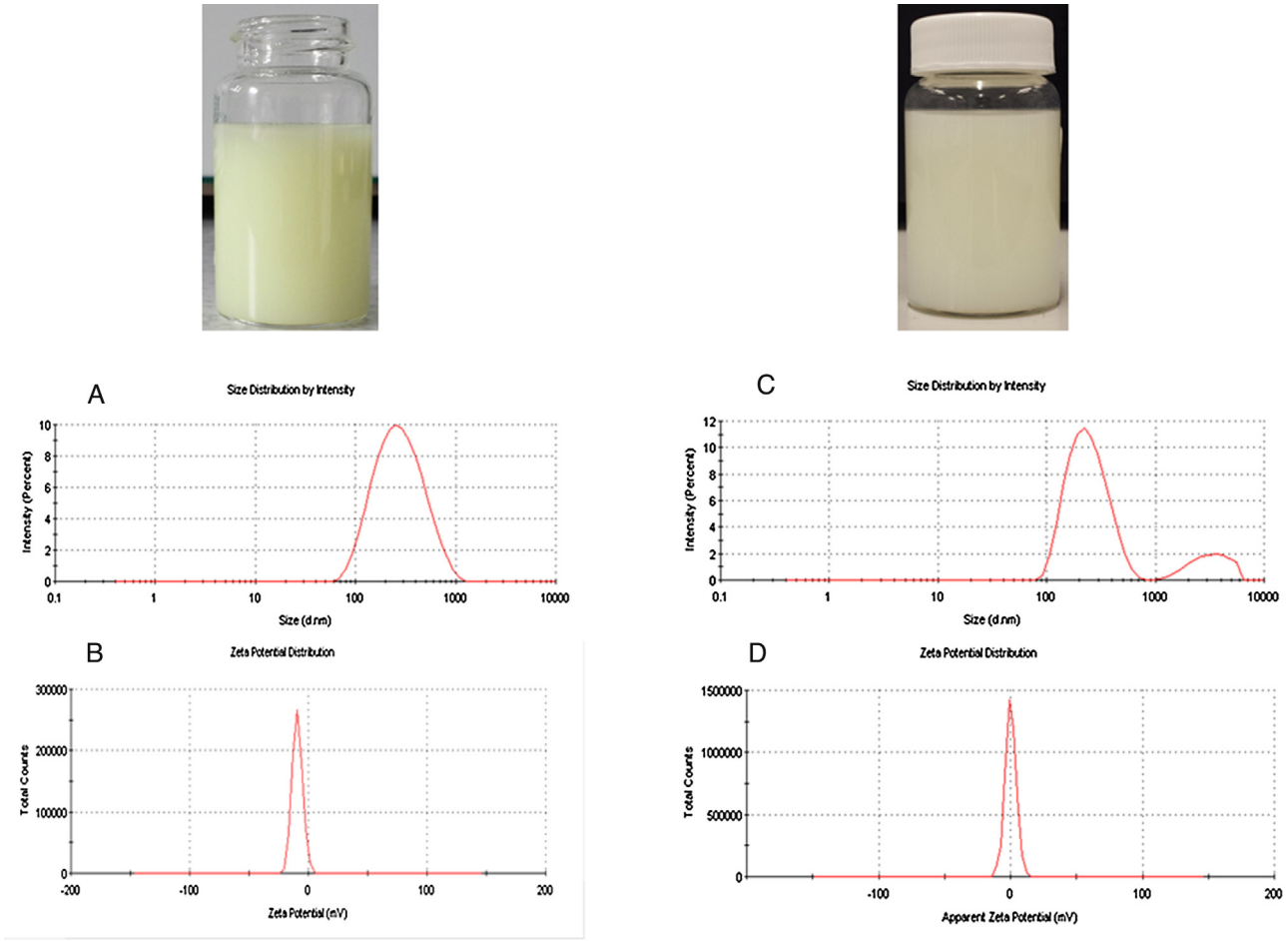

Fig. 1. RTA-SLNs made by stearic acid: (a) particle size distribution (nm), (b) zeta potential (mV). RTA-SLNs made by tristearin (c) Particle size distribution of RTA-TS SLNs (d) zeta potential (mV) (size quoted as z-average mean (nm) of the hydrodynamic diameter (nm).