International Journal of Pharmaceutics 471 (2014) 18–27

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

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

Pharmaceutical nanotechnology

Cationic solid lipid nanoparticles interfere with the activity of antioxidant enzymes in hepatocellular carcinoma cells

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ARTICLE INFO

Article history:
Received 8 March 2014
Received in revised form 7 May 2014
Accepted 8 May 2014
Available online 13 May 2014

Keywords:
Solid lipid nanoparticles
Oxidative stress
HepG2 cells
DCFDA
Glutathione reductase
TBARS

ABSTRACT

Solid lipid nanoparticles (SLN) are colloidal drug and/or gene carriers developed from solid lipids and surfactants that are considered safe. Cationic SLN, usually used for formulating poorly water-soluble drugs and for gene delivery purposes, as positively charged particles may attach to cellular surfaces and be internalized more easily than negatively charged SLN, but they can also cause damage. The main aim of this work was to test a set of cationic SLN and investigate its influence on the amount of reactive oxygen species (ROS), on antioxidant enzymes activities and on possible oxidative damage to membrane lipids in HepG2 cells. The Dichlorofluorescein assay revealed great increase in ROS presence after cell exposure to SLN. While the exposure to SLN increased the activities of superoxide dismutase and glutathione peroxidase it decreased glutathione reductase activity. Although no significant increase in thiobarbituric reactive species was found, a decrease in sulfhydryl groups was detected. These results indicate that cationic SLN caused oxidative stress in HepG2 cells, but under reported exposure conditions HepG2 cells could attenuate the stress and thus the damage to cellular components was minimal.

1. Introduction

Reactive oxygen species (ROS) are species of oxygen with unpaired electron, e.g., superoxide radical ($O_2^{\bullet -}$), hydroxyl, peroxyl or alcoxyl radicals. ROS occur in living cells under physiological conditions. Main source of ROS is oxidative phosphorylation, to smaller extent they can be generated by activity of other enzymes (e.g., 5-lipoxygenase, cyclooxygenase, xanthine oxidase), cytochrome P450 (Hayes and McLellan, 1999) or by auto-oxidation of cell components (Santos et al., 2007). The level of intracellular ROS is linked to regulation of apoptosis (Chandra et al., 2000), protein/protein disulphide bond formation and cell signalling (Filomeni et al., 2002). Increased ROS levels lead to oxidation of membrane lipids (which can further propagate and generate more lipoperoxides), damage of proteins (depletion of protein sulfhydryl groups) and damage of DNA. The condition of increased ROS levels are referred to as oxidative stress and is considered to contribute to pathologic conditions including, among others, neurodegenerative diseases or carcinogenesis (Hayes and McLellan, 1999).

Cellular defence against oxidative stress includes non-enzymatic antioxidants such as glutathione, thioredoxyn, carotens, tocopherols or ubiquinone; and enzymatic antioxidants – superoxide dismutase (SOD, EC 1.15.1.1) which metabolises superoxide radicals into H_2O_2, catalase (EC 1.11.1.6) and glutathione peroxidase (Gpx, EC 1.11.1.9) that processes H_2O_2, glutathione reductase (GR, EC 1.8.1.7) which regenerates reduced glutathione (GSH) from its oxidized form (GSSG) and glutathione S-transferase (GST, EC 2.5.1.18) which catalyses GSH association with various compounds and regenerates NADPH pool. The action of these enzymes is schematically depicted in Fig. 1.

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http://dx.doi.org/10.1016/j.ijpharm.2014.05.011
0378-5173/

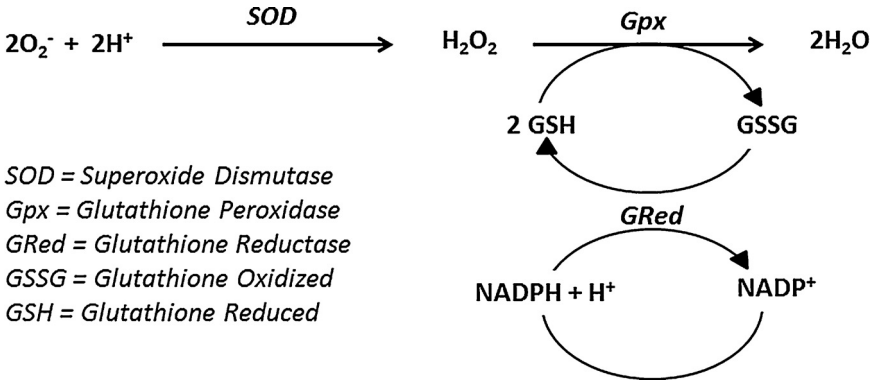

Fig. 1. Scheme of action of antioxidant enzymes and their cooperation in the course of free radicals deactivation (e.g., superoxide anion).

Solid lipid nanoparticles (SLN), colloidal systems (diameters <1 μm) composed of lipids that are solid at room temperature, have been proposed as drug delivery system for poorly soluble drugs (Souto and Müller, 2007). These systems are by definition composed from materials already approved for use in pharmaceutical or cosmetic products, such as triacylglycerides, diacylglycerides, monoacylglycerides, waxes or fatty acids and surfactants accepted for use in medicines (Müller et al., 2011). SLN offer the possibility to obtain a drug delivery system which releases the drug at a controlled rate, can be readily prepared by methods feasible for industrial-scale production, and is prepared from inexpensive starting materials. Furthermore, various authors reported improved intracellular delivery of encapsulated drugs (Lin et al., 2013; Zhu et al., 2009), including chemotherapeutic drugs, and reversal or cancer cell line resistance when the chemotherapeutic active was administered in SLN (Zhang et al., 2008; Zhang et al., 2012).

Cationic SLN (cSLN) contain a cationic lipid/surfactant and are used either for gene delivery or for drug delivery for special routes, e.g., to eye (Souto et al., 2010). Cationic SLN are also developed in special cases of drugs that are poorly soluble in water as well as lipids to improve encapsulation efficiency (Castro et al., 2009; Kuo and Liang, 2011). In comparison to traditional SLN with negative surface charge, positively charged SLN (and other types of colloidal carrier in general) have greater affinity to serum proteins (Lv et al., 2006) and cell membranes. The latter was proposed to facilitate interaction with cellular receptors and subsequent uptake. Indeed, higher quantity of drug internalized in skin cells was found when the drug was encapsulated in positively charged nanostructured lipid carriers (NLC, a modification of solid lipid nanoparticles which includes further oil component and provides higher stability) than in negatively charged NLC (Lin et al., 2013).

In this work, we tested a previously developed series of cSLN for possible interference with cellular mechanisms of ROS level control. A non-toxic concentration and a toxic (viability <70%) concentration of cSLN were tested in HepG2 cell line. This human hepatocarcinoma cell line is used as a model of hepatotoxicity since the metabolism of normal hepatocytes is preserved. We focused mainly on activity of ROS-metabolising enzymes SOD and Gpx and also examined GR and GST activity. In order to verify if cSLN influence the level of ROS, 2′,7′-dichlorodihydrofluorescein-diacetate ($DCFH_2$-DA) assay was used to obtain an estimative of radical species present in cells. We also determined the amount of thiobarbituric acid reactive species (TBARS), which give an estimative of oxidized lipid compounds and protein sulfhydryl (thiol) groups to find out any damage to cellular components that might have occurred.

2. Material and methods

2.1. Material

Solid lipids (Imwitor 900K, Compritol 888 ATO) for cSLN preparation were obtained from Sasol GmbH, Germany and Gattefosse, S.A., France, respectively. Imwitor 900K (IMW) is a mixture containing 40–55% glycerylmonostearate and 30–45% glyceryldistearate. Compritol 888 ATO contains mainly glyceryldibehenate (40–60%), further contains glyceryltribehenate (21–35%) and monobehenate (15–23%). Cetyltrimethylammonium bromide (CTAB), Lutrol F68 (Poloxamer 188), Miranol C-32 Ultra were obtained from Sigma Portugal, Sasol GmbH Germany and Croda GmbH, Germany, respectively.

Reagents for cell culture were obtained from Gibco (Alfagene, Portugal). Alamar Blue from Invitrogen Alfagene, Portugal was used for cell viability estimation.

Trichloracetic acid was from AppliChem. All other reagents (for determination of enzyme activity, oxidized lipids content and oxidized proteins were obtained from Sigma: $NADPH^+H^+$, GSH, GSSH, NBT (3,3′-(3,3′-dimethoxy [1,1′-biphenyl]-4,4′-diyl) bis [2-(4-nitrophenyl)]-2H-tetrazolium dichloride), CDNB (1-chloro-2,4-dinitrobenzene), hypoxanthine, glutathione reductase, xanthine oxidase, DCF-DA (dichlorofluorescein-diacetate), thiobarbituric acid (TBA), BHT (2,6-di-*tert*-butyl-4-methylphenol/butylatedhydroxytoluene), DTNB (5,5'-ditiobis(2-nitrobenzoate), sulfosalicilyc acid, BSA (bovine serum albumin) and salts for buffer solutions.

2.2. Equipment

Absorbance readings were performed on Varian Cary 50 or Varian Cary 100 using Kinetics mode for enzyme kinetics (Varian). Absorbance reading for protein quantification, TBARS quantification and cell viability evaluation were recorded on a Multiskan EX microplate reader (MTX LabSystems, USA). For estimation of oxidized proteins, absorbance was recorded using a BioTekPowerWave X52 microplate reader.

2.3. Methods

2.3.1. SLN production and characterization

The cSLN were produced as described before (Doktorovova et al., 2014). Composition of cSLN is presented in Table 1. The intensity-weighted average diameter (*z*-average, *z*-ave) of original aqueous cSLN dispersions was determined by photon correlation spectroscopy using a Zetasizer Nano ZS (Malvern Instruments, CA, USA). Polydispersity indices (PdI) were derived