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High payload nanostructured lipid carriers fabricated with alendronate/polyethyleneimine ion complexes

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Chemical compounds studied in this article:
Alendronate sodium (CID: 16760285)
Polyethyleneimine (SID: 316469240 [CID: 9033])
Medium chain caprylic/capric triglyceride mixture (USP 29 – NF 24 Page 3452)
Precirol® ATO-5 (CID: 114690)

ABSTRACT

Oral bioavailability of the anti-osteoporotic drug alendronate (AL) is limited to ≤ 1% due to unfavorable physicochemical properties. To augment absorption across the gastrointestinal mucosa, an ion pair complex between AL and polyethyleneimine (PEI) was formed and incorporated into nanostructured lipid carriers (NLCs) using a modified solvent injection method. When compared to free AL, ion pairing with PEI increased drug encapsulation efficiency in NLCs from 10% to 87%. Drug release from NLCs measured *in vitro* using fasted state simulated intestinal fluid, pH 6.5 (FaSSIF-V2) was significantly delayed after PEI complexation. Stability of AL/PEI was pH-dependent resulting in 10-fold faster dissociation of AL in FaSSIF-V2 than measured at pH 7.4. Intestinal permeation properties estimated *in vitro* across Caco-2 cell monolayers revealed a 3-fold greater flux of AL encapsulated as hydrophobic ion complex in NLCs when compared to AL solution (P_{app} = 8.43 ± 0.14 × 10^{-6} cm/s and vs. 2.76 ± 0.42 × 10^{-6} cm/s). Cellular safety of AL/PEI-containing NLCs was demonstrated up to an equivalent AL concentration of 2.5 mM. These results suggest that encapsulation of AL/PEI in NLCs appears a viable drug delivery strategy for augmenting oral bioavailability of this clinically relevant bisphosphonate drug and, simultaneously, increase gastrointestinal safety.

1. Introduction

Postmenopausal osteoporosis is one of the most common skeletal disorders estimated to affect 25% of the female population over 50 years old (Kassem and Marie, 2011). Pathophysiologically, osteoporosis represents an imbalance in bone homeostasis driven by increased bone resorption, which decreases bone mineral density and bone microarchitecture. As a consequence, patients have an increased risk of fractures (Cummings and Melton, 2002). Nitrogen-containing bisphosphonate drugs such as alendronate sodium (AL) are generally used as first-line intervention in osteoporosis due to their ability to inhibit bone resorption mediated by osteoclasts (Sparidans et al., 1998). After oral administration, unfavorable polar molecular properties limit absorption of bisphosphonates across the gastrointestinal mucosa resulting in oral bioavailabilities of 1% or less (Watts and Diab, 2010). Moreover, commercial oral formulations of bisphosphonates are reported to induce severe irritation of the upper gastrointestinal tract, including mucosal inflammation and erosion. To reduce the risk of these adverse events, patients have to comply with a draconian dosing regimen requiring co-administration of large volumes of fluid followed by period of at least 30 min in upright position without ingestion of food (Gertz et al.,1993; Watts and Diab, 2010).

Several drug delivery strategies have been explored to enhance safety and efficacy of oral bisphosphonates. Baek and co-workers demonstrated that AL-containing microparticles formulated using chitosan as mucoadhesive polymer and Eudragit L100-55 as a gastric-resistant polymer increase intestinal absorption 3-fold (Baek et al., 2011). Similarly, oral bioavailability of AL was enhanced 2.6-fold when formulated as mucoadhesive, chitosan-coated liposomes (Han et al., 2012). Sakuma and colleagues prepared poly(vinylamine)-alendronate conjugates using a Suc-Gly-Gly-Phe-Ala-Ala-Pro spacer, which increased oral AL absorption by 2.5-fold (Sakuma et al., 2007).

Abbreviations: AL, alendronate sodium; PEI, polyethyleneimine; NLC, nanostructured lipid carrier; FASSIF-V2, fasted state simulated intestinal fluid; HBSS, Hanks' balanced salt solution; HEPES, N-[2-hydroxyethyl] piperazine-N- [2-ethanesulfonic acid]; ATCC, American Type Culture Collection; DMEM, Dulbecco's modified Eagle medium; MCT, medium chain caprylic/capric triglyceride mixture; FBS, fetal bovine serum; PBS, phosphate buffered saline; MTT, (4,5-dimethylthiazol-2-yl)2,5-diphenyl-tetrazolium bromide; RT, room temperature; DLS, dynamic laser light scattering; ITC, isothermal titration calorimetery; EE%, encapsulation efficiency; LC, loading capacity; Papp, apparent permeability coefficient

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Nanostructured lipid carriers (NLCs) represent a new generation of solid lipid nanoparticles that are produced by controlled mixing of solid lipids with spatially incompatible liquid lipids. Incorporation of a liquid lipid fraction disrupts the crystal lattice of the solid lipid and, thus, creating matrix imperfections that enable increased drug loading (Davda and Labhasetwar, 2002). Similar to solid lipid nanoparticles, NLCs are biocompatible, biodegradable, and suitable for controlled release drug delivery applications for drug molecules exhibiting predominantly lipophilic physicochemical properites (Jia et al., 2010; Müller et al., 2002). However, limited affinity of hydrophilic drugs for the lipid components generally used to fabricate NLCs results into unsatisfactory loading capacities for water-soluble drugs in these nanosized lipophilic carriers (El-Salamouni et al., 2015; Selvamuthukumar and Velmurugan, 2012). To augment NLC loading efficiency for hydrophilic drugs, electrostatically stabilized association complexes with hydrophobic counter ions can be formed. These non-covalent ion pairs transiently enable greater affinity with lipid excipients and, hence, favor preferential partitioning into the lipid matrix during NLC fabrication. Successful examples of this hydrophobic ion pairing strategy resulting in increased NLC encapsulation efficiency are reported for doxorubicin, verapamil, and quinidine, respectively (Oliveira et al., 2016a,b; Wong et al., 2004).

The objective of this research was to quantitatively assess the consequences of hydrophobic ion pairing of AL with polyethyleneimine (PEI) on the encapsulation efficiency of this hydrophilic bisphosphonate drug within NLCs comprised of Precirol® ATO-5 and medium chain triglycerides. Furthermore, it was determined whether encapsulation of the hydrophobic AL/PEI ion complex within this lipid-based nanodelivery system successfully enhances the flux of this highly polar drug across the intestinal mucosa *in vitro* using the Caco-2 cell culture model. Finally, it was attempted to delineate the influence of the gastrointestinal environment on the release kinetics of AL and AL/PEI ion pairs from NLCs.

2. Materials and methods

2.1. Materials

Alendronate sodium, glacial acetic acid, sodium acetate trihydrate and *N*-[2-hydroxyethyl] piperazine-*N*-[2-ethanesulfonic acid] (HEPES) were obtained from Sigma Aldrich (St. Louis, MO). Polyethyleneimine (branched, average M_W = 25 kDa), perchloric acid 70% (w/v) solution in water, ferric chloride hexahydrate, Ca^{2+}- and Mg^{2+}-free Hanks' balanced salt solution (HBSS), Dulbecco's Modified Eagle Medium (DMEM), phosphate buffered saline (PBS), and dimethyl sulfoxide and 4,5-dimethylthiazol-2-yl)2,5-diphenyl-tetrazolium bromide (MTT) were purchased from Fisher Scientific (Pittsburgh, PA). Precirol® ATO-5 and the medium chain caprylic/capric triglyceride mixture (MCT) were a kind gift from Gattefossée (Paramus, NJ). Poloxamer® 407 was purchased from BASF (Greenville, OH). Fasted state simulated intestinal fluid (FaSSIF-V2) was obtained from Biorelevant.com (Croydon, Surrey, UK). Fetal bovine serum (FBS) was obtained from Atlanta Biologicals (Flowery Branch, GA). *L*-glutamine 200 mM (100x), penicillin/streptomycin (10000 IU/ml and 10000 µg/ml, respectively), and non-essential amino acids 10 mM (100x) were obtained from Invitrogen (Carlsbad, CA). [^{14}C]Alendronate (specific activity = 49.7 mCi/mmol) and [^{14}C]mannitol (specific activity = 58 mCi/mmol) were purchased from Moravek Biochemicals (Brea, CA). Rat tail collagen type I was obtained from Collaborative Biomedical Products (Bedford, MA). All other reagents were of analytical grade and used without further purification.

2.2. Methods

2.2.1. AL/PEI ion pair formation

PEI was dissolved at room temperature (RT) in 1% (w/v) acetic acid resulting in 152 pM − 152 µM acidic PEI solutions. Three mL of the PEI solution were added drop-wise to an equivalent volume of a 1 mM AL solution prepared in 10 mM acetate buffer, pH 5.0. The mixture was stirred for 1 min at RT followed by a 45 min centrifugation at 4000 × *g* to remove high molecular weight aggregates. Electrostatically stabilized AL/PEI association complexes were collected in the supernatant, and physicochemical properties such as particle size distribution and zeta potential were measured by dynamic laser light scattering (DLS, see below). To determine complexation efficiency at different molar AL/PEI ratios, the concentration of free AL was quantified spectrophotometrically at λ = 300 nm using the colored ferric/AL complex formed in 0.01 M perchloric acid, pH 2, as described previously by this laboratory (Abd El-Hamid et al., 2015).

2.2.2. Isothermal titration calorimetry

Thermodynamic properties of AL/PEI interactions in 10 mM acetate buffer, pH 5, were measured by isothermal titration calorimetery (ITC) using the Nano-ITC 2G calorimeter (TA Instruments, New Castle, DE). Titrations were carried out at 25 ± 0.02 °C by injecting 25 aliquots of 10 µL each of a 1.245 mM AL solution in acetate buffer, pH 5. The sample cell was filled with a 641.5 nM PEI solution prepared in the same buffer solution. The heat of reaction was measured after each injection and plotted against the AL ligand concentration . Nonlinear regression analysis of baseline-corrected calorimetric data was employed using Nanoanalyze 3.5.0. (TA Instruments, New Castle, DE) to estimate thermodynamic binding parameters, including the binding constant (K_a), enthalpy (ΔH), and number of binding sites (n), respectively. To assess whether release of the pharmacologically active AL from its ion complex with PEI is thermodynamically feasible after absorption into the cardiovascular systems, binding properties between AL and PEI were also measured by ITC using Hanks' balanced salt solution, pH 7.4 (HBSS).

2.2.3. Particle size and zeta potential

Particle size distribution of electrostatically stabilized AL/PEI complexes and fabricated NLCs was determined at 25 °C by dynamic laser light scattering (DLS) using the Malvern Zetasizer Nano-ZS (Malvern Instruments, Worcestershire, UK) equipped with a 4 mW helium/neon laser (λ = 633 nm). All particle size values reported in this study correspond to hydrodynamic diameters. The zeta potential of the colloids was estimated using electrophoretic mobility data acquired in 10 mM acetate buffer, pH 5.0.

2.2.4. Fabrication of drug-loaded nanostructured lipid carriers

AL or AL/PEI ion complexes (6.6:1, mol/mol) prepared in 10 mM acetate buffer, pH 5, were encapsulated into NLCs using the solvent injection method as described by Chen et al. (2012) with the following modifications (Chen et al., 2012). Briefly, 100 mg of a Precirol® ATO-5 and MCT mixture (3:1 w/w) were dissolved at 60 °C in 2 mL of an acetone/ethanol mixture (3:1, v/v) to form the oil phase. The aqueous phase (30 mL) was comprised of AL or AL/PEI complexes in the presence of 1% (w/v) Poloxamer® 407. The oil phase was cooled to approximately 27–30 °C and rapidly injected into the aqueous phase under stirring (1000 rpm). Organic solvents were evaporated at room temperature under stirring. Particle suspension was sonicated for 20 min sonication before physicochemical properties were measured by DLS. As a control, AL-free placebo NLCs were prepared using the identical fabrication protocol.

Drug encapsulation efficiency (EE%) and loading capacity (LC) of AL or AL/PEI complexes in NLCs were determined indirectly by measuring unincorporated AL spectrophotometrically at λ = 300 nm after ultrafiltration (Amicon® Ultra-4 100k, EMD Millipore, Billerica, MA) using ferric chloride in 0.2 M perchloric acid, pH 0.7. Calculations were performed according to: