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Enhanced solubility and modified release of poorly water-soluble drugs via self-assembled gelatin–oleic acid nanoparticles

Phuong Ha-Lien Tran [a], Thao Truong-Dinh Tran [a], Beom-Jin Lee [b,*]

[a] International University, Vietnam National University – Ho Chi Minh City, Viet Nam
[b] College of Pharmacy, Ajou University, Suwon 443-749, Republic of Korea

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ABSTRACT

Recently, we synthesized novel amphiphilic gelatin–oleic acid (GO) conjugate to prepare self-assembled nanoparticles for drug delivery. The aim of this study was to investigate pharmaceutical potentialities of self-assembled GO nanoparticles for solubility enhancement and modified release of poorly water-soluble drugs. Three poorly water-soluble model drugs with different pH-dependent solubility (valsartan and aceclofenac, insoluble at pH 1.2; telmisartan, insoluble at pH 6.8) were chosen to investigate the potential contributions of self-assembled GO nanoparticles to solubility enhancement and controlled release. The particle size of the drug-loaded nanoparticles was 200–250 nm. Zeta potential was calculated, and instrumental analysis such as powder X-ray diffraction (PXRD) and Fourier transform infrared (FT-IR) spectroscopy were used to investigate the physicochemical properties of the drug-loaded nanoparticles. Compared to the drug alone, the drug-loaded nanoparticles showed enhanced solubility. Furthermore, the release profiles of the model drugs were modified in a controlled manner. The current self-assembled GO nanoparticles can provide a versatile potential in drug delivery and tumor targeting.

1. Introduction

Nanomaterial has become an interesting subject of research in many fields including environment, electronics, information and communication, and medicine. These materials are expected to have a major impact in the medical field because of having the size between the largest biological molecules and the smallest manmade devices. The use of nanoparticles as drug delivery systems is gaining popularity because of a number of advantages such as placing nano-objects at the desired position, increasing the bioavailability of drugs, enhancing solubility and controlling the drug release rate. Nanoparticles applied as drug delivery devices or systems are submicron-sized particles (3–200 nm) that can be made using a variety of materials including polymers (polymeric nanoparticles, micelles, or dendrimers), lipids (liposomes), viruses (viral nanoparticles), and even organometallic compounds (nanotubes) (Cho et al., 2008). Regarding polymer-based drug carriers, natural polymers such as albumin, chitosan, gelatin, and heparin have been used for the delivery of oligonucleotides, DNA, proteins, and drugs. In addition to synthetic polymers such as *N*-(2-hydroxypropyl) methacrylamide copolymer (HPMA), polystyrene-maleic anhydride copolymer, polyethylene glycol (PEG), and poly-L-glutamic acid, biomaterials such as polyglycolic acid (PGA), polycyanoacrylate, poly-D,L-lactide, polylactic acid, or poly(lactide-co-glycolide) have been introduced as nano drug delivery systems (Kayser et al., 2005; Cho et al., 2008).

However, recent researches on various self-assembling delivery systems such as polymeric nanoparticles and branched amphiphilic peptides (surfactant-like peptide) capable of forming nanotubes and nanovesicles have promises in substituting conventional nanoparticles for cellular targeting and multifunctional intelligence of drugs (Motornov et al., 2010; Gudlur et al., 2012). Most of all, a multifunctional nanoparticulate system capable of enhancing the solubility of poorly water-soluble drugs, controlling drug release rates and modifying bioavailability would be more preferable in drug delivery and pharmaceutical applications.

Recently, we originally developed a novel amphiphilic gelatin–oleic acid (GO) conjugate and investigated its potential pharmaceutical applications in drug delivery by forming self-assembled nanoparticles (Tran et al., 2013a,b). In pharmaceutics, biodegradable and biocompatible polymeric nanoparticles have shown great potential as drug carriers. Gelatin is a naturally biodegradable macromolecule with well-documented biocompatible properties over other synthetic polymers, making it a suitable material for use as a nanoparticulate carrier (Lai et al., 2006). Furthermore, gelatin is inexpensive and readily available for various chemical modifications, because gelatin has a primary structure containing functional groups and the presence of multifunctional

* Corresponding author at: Bioavailability Control Laboratory, College of Pharmacy, Ajou University, Suwon 443-749, Republic of Korea.
Tel.: +82 31 219 3442; fax: +82 31 212 3653.
E-mail addresses: beomjinlee@gmail.com, bjl@ajou.ac.kr (B.-J. Lee).

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charged groups and hydrophobic regions in gelatin molecules (Fitch et al., 1969; Li et al., 1998; Bajpai and Choubey, 2006). Oleic acid, a biocompatible and biodegradable fatty acid, is also used as a stability-inducing agent for many nanoparticle systems (Hosokawa et al., 2002; Ledo-Suárez et al., 2006).

In this work, oleic acid was selected to conjugate with gelatin by using a very simple method with monoethanolamine (MEA) in water to form a biocompatible, biodegradable, and stable nanoparticle that self-assembles upon dispersion in an aqueous medium. This GO nanoparticle was applied as a carrier to investigate solubility and dissolution rate in a controlled-release fashion for three model drugs such as valsartan (VAL) and aceclofenac (AFC), which have low solubility in simulated gastric fluid (pH 1.2) and telmisartan (TEL), which has low solubility in simulated intestinal fluid (pH 6.8). Particle size distribution, zeta potential, powder X-ray diffraction (PXRD), and Fourier transform infrared (FT-IR) spectroscopy were used to characterize the physicochemical properties of the drug-loaded nanoparticles.

2. Materials and methods

2.1. Materials

Gelatin was purchased from Kanto Chemical Co., Inc. (Tokyo, Japan). Oleic acid was purchased from Shinyo Pure Chemicals Co., Ltd. (Osaka, Japan). MEA was purchased from Yakuri Pure Chemicals Co., Ltd. Telmisartan and Valsartan were purchased from NJMMM Co. (Nanjing, China) and Du-Hope Pharmaceutical Corp (Nanjing, China), respectively. Aceclofenac was obtained from Dae Woong Pharmaceutical Co. Ltd, (Seoul, Korea). The solvents used were high-performance liquid chromatography (HPLC) grade. All other chemicals were of analytical grade and were used without further purification.

2.2. Methods

2.2.1. Preparation of drug-loaded GO nanoparticles

GO conjugates previously synthesized (Tran et al., 2013a) and the drug (AFC, VAL, or TEL) were dispersed in dichloromethane (loading amount of drug was 10%). The solution (300 μL) was then emulsified into 10 mL of distilled water and sonicated for 20 min to form an oil/water emulsion. Dichloromethane was then evaporated under purged nitrogen gas for 15 min. The large aggregates, if present, were removed by centrifugation at 1000 rpm for 5 min at 37 °C. Nanoparticles were then harvested and washed 3 times with distilled water by centrifugation at 40,000 rpm for 20 min at 37 °C. The pellets were resuspended in water, sonicated for 30 s, and lyophilized and freeze-dried at −50 °C for 2 days. Drug-loaded nanoparticles were labeled as VAL-GO, AFC-GO, or TEL-GO, corresponding to the drugs encapsulated in the GO nanoparticles.

2.2.2. HPLC analysis

After gathering the supernatant and washings collected from the nanoparticle preparation, the drug loading content and encapsulation efficiency in the nanoparticles were determined indirectly by HPLC analysis (Waters™, USA) with a reversed-phase column (150 × 4.6 mm, Luna 5u C18 100 A) and injection volume of 20 μL. For AFC analysis, the mobile phase was a mixture of 20 mM phosphate buffer and methanol (35:65, v/v); the flow rate was 1.2 mL/min and the detection wavelength was 282 nm. For VAL analysis, the mobile phase consisted of 20 mM phosphate buffer (pH 2, adjusted by phosphoric acid) and acetonitrile (45:55, v/v); the flow rate was 1.2 mL/min, and the detection wavelength was 234 nm. For TEL analysis, the mobile phase consisted of a 75:25 (% v/v) mixture of methanol and 51.8 mM ammonium acetate; the flow rate was 1.0 mL/min, and the detection wavelength was 296 nm.

2.2.3. In vitro drug release

Drug-loaded nanoparticles were dispersed in 10 mL of simulated gastric fluid (pH 1.2; for VAL and AFC) and simulated intestinal fluid (pH 6.8; TEL) in screw-capped tubes, and the tubes were placed in an orbital shaker maintained at 37 °C and shaken at 100 rpm. The amount of nanoparticles in 10 mL of media was calculated to be equivalent to the drug amount in dosage form tested in 900 mL of media under the conventional apparatus of a dissolution tester (70 mg for AFC, and 80 mg for VAL and TEL). At predetermined time intervals, the tubes were taken out and centrifuged at 40,000 rpm for 20 min. The supernatant was saved for HPLC analysis to determine the drug release. The precipitated pellets were resuspended in 10 mL of fresh buffer and placed back in the shaker.

2.2.4. Solubility study

The solubilities of the pure drugs and the drugs loaded in the nanoparticles were determined in simulated gastric fluid (pH 1.2) (VAL, AFC) and simulated intestinal fluid (pH 6.8) (TEL) by adding excess amounts of the drugs to snap-cap Eppendorf tubes (Hamburg, F.R.G) containing 1 mL of media. The resulting mixture was sufficiently vortexed and then placed in an incubator at 37 °C for 2 days. Aliquots were centrifuged at 15,000 rpm for 10 min. The supernatant layer was carefully collected and diluted with a solvent for the mobile phase in the HPLC analysis, based on preliminary solubility tests. The drug concentration was then quantified by HPLC from a standard calibration curve.

2.2.5. Particle size measurements and zeta potential

The average particle size of the self-assembled nanoparticles was measured using a PAR-III Laser Particle Analyzer System (Otsuka Electronics, Japan). All measurements were performed in triplicate by using a He-Ne laser light source (5 mW) at a 90° angle.

The zeta potential of nanoparticles was calculated using an Electrophoretic Light Scattering Spectrophotometer 8000 (Otsuka Electronics, Japan) at −28.3 V/cm, −0.1 mA, and 28 °C.

The sample concentration was maintained at 1 mg/mL in distilled water.

2.2.6. Morphology of the nanoparticles

A solution of the self-assembled nanoparticles (1 mg/mL) was dropped onto a copper grid to observe the morphology with transmission electron microscopy (TEM; LEO 912AB-100, Carl Zeiss, Korea Basic Science Institute-Chuncheon). After drying in a vacuum dryer at room temperature, the grid was examined using the transmission electron microscope.

2.2.7. Fourier transform infrared spectroscopy (FT-IR)

The spectra of the samples (Gelatin, OA, conjugates, VAL-GO, AFC-GO, and TEL-GO) were recorded using an IR spectrophotometer (BIO-RAD, USA MODEL EXCALIBER Series UMA-500). KBr pellets were prepared by gently mixing 1 mg of the sample with 200 mg of KBr. FT-IR (400–4000 cm^{-1}) was performed with a resolution of 2 cm^{-1}.

2.2.8. Powder X-ray diffraction (PXRD)

PXRD patterns of the samples (Gelatin, OA, conjugates, VAL-GO, AFC-GO, and TEL-GO) were analyzed using a D5005 diffractometer (Bruker, Germany) using Cu Kα radiation at a voltage of 40 kV and a current of 50 mA. The powder samples were scanned in 0.02° steps from 5° to 60° (diffraction angle 2θ) at a rate of 1 s per step by using a zero background sample holder.