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Preparation of an injectable depot system for long-term delivery of alendronate and evaluation of its anti-osteoporotic effect in an ovariectomized rat model

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ABSTRACT

We prepared an injectable depot system for the long-term delivery of alendronate using a solid/water/oil/water multiple emulsion technique with poly(lactic-co-glycolic acid) as a carrier. The microparticles were spherical with smooth surfaces, ranging from 20 to 70 μm in size. The microspheres (ALD-HA-RG504H-MC70) were optimally prepared by introducing a viscous material (hyaluronic acid) and a co-solvent system in the inner aqueous and oil phases, respectively, and showed a significantly increased drug encapsulation efficacy (>70%); the initial burst release was <10% after 1 day. *In vitro* drug release from ALD-HA-RG504H-MC70 followed zero-order kinetics for approximately 4 weeks and the alendronate plasma level was maintained for more than 1 month after intramuscular injection in rabbits. The ovariectomized (OVX) rats with ALD-HA-RG504H-MC70 injected intramuscularly (0.9 mg alendronate/kg/4 weeks) had 112% and 482% increased bone mineral density and trabecular area in the tibia than the OVX controls, respectively, and showed significant improvements in trabecular microarchitecture and bone strength. Furthermore, the major biomarkers of bone turnover revealed that ALD-HA-RG504H-MC70 suppressed effectively the progression of osteoporosis and facilitated new bone formation. Therefore, this sustained release depot system may improve patient compliance and therapeutic efficacy by reducing dose amounts and frequency with minimal adverse reactions.

1. Introduction

Osteoporosis is one of the most prevalent metabolic diseases affecting bone. It is characterized by low bone mass and micro-architectural deterioration of bone tissue, caused by an imbalance between bone formation and absorption (Allen, 2008). This chronic disease increases susceptibility to osteoporotic bone fractures and postural deformities. In particular, fractures of the hips or vertebrae are closely associated with significant mortality. Osteoporosis is typically more common in postmenopausal women because of an acceleration of bone loss. However, it can occur in men and women, and is becoming a serious public health problem due to the increasing older population (Rizzoli et al., 2001).

Bisphosphonates such as alendronate are potent, specific inhibitors of osteoclast-mediated bone resorption that modulate bone metabolism (Coxon et al., 2006; Reszka and Rodan, 2003). Thus, they have been used clinically to treat systemic metabolic bone diseases, such as osteoporosis, Paget's disease, hypercalcemia of malignancy, and inflammation-related bone loss (Fisher et al., 1999; Im et al., 2004).

However, bisphosphonates have fairly low oral bioavailability (<1%) due to their high polarity and hydrophilicity, resulting in low gastrointestinal (GI) permeability (Lin et al., 1994). Furthermore, patients taking oral bisphosphonate need to make sure that they take it after an overnight fast, and remain upright for at least 30 min post-dose because of its severe interaction with foods and side effects, such as esophagitis, abdominal pain, and acid reflux (Adami and Zamberlan, 1996; Blumentals et al., 2009; Gertz et al., 1993; Peter et al., 1998).

To overcome these disadvantages and provide better patient compliance, many formulation modifications have been conducted, and most of them have focused on improving the oral bioavailability, reducing side effects, or increasing dose interval (Cramer et al., 2005; Ezra et al., 2000; Janner et al., 1991). Another change is in the mode of administration, such as intravenous (i.v.) infusion, intramuscular, or subcutaneous injection by pulmonary

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or transdermal delivery routes, because this can significantly reduce the dose, and then the therapeutic effect can easily be prolonged by increasing the dose (Ezra and Golomb, 2000; Miladi et al., 2013; Nam et al., 2012). However, rapid injection of a bisphosphonate can cause complex formation with calcium in the blood, which can then lead to renal failure by being retained in the kidney (Ezra and Golomb, 2000). In addition, local delivery of bisphosphonates can increase the risk of tissue irritation or damage (Lin, 1996). Thus, there have been various attempts to incorporate the bisphosphonate into a sustained release carrier to provide a long-acting injectable or inhalable delivery system.

To prepare injectable sustained release systems, bisphosphonates have been encapsulated with biodegradable polymeric materials, such as poly(lactic-co-glycolic acid) (PLGA), poly (lactic-co-caprolactone), Eudragit L100-55, calcium phosphate, poly(lactic acid), poly(D,L-lactic acid)-block-poly(ethylene glycol), and poly(β-hydroxybutyrate-co-β-hydroxyvaleate) through oil-in-water (o/w) or water-in-oil-in-water (w/o/w) micro-emulsions with solvent evaporation methods (Balas et al., 2006; Nafea et al., 2007; Patashnik et al., 1997; Umeki et al., 2010). However, the bisphosphonates are quite easily lost in the aqueous phase during the encapsulation process and quickly diffuse out from the carrier, resulting in significantly low drug encapsulation efficiency and a great initial burst release (Miladi et al., 2013). To overcome these limitations, several studies have ionically complexed the bisphosphonate with hydroxyapatite, or used an osmogen in the external aqueous phase (Nasr et al., 2011; Shi et al., 2009a,b).

The purpose of this study was to fabricate an injectable depot system for the long-term delivery of bisphosphonates. In this study, we used alendronate as a model bisphosphonate, and prepared controlled-release microspheres by a solid/water/oil/water (s/w/o/w) multiple emulsion technique using PLGA as the carrier. Furthermore, we introduced a hydrophilic viscous material, such as hyaluronic acid, in the inner aqueous phase and used a co-solvent system in the organic phase to increase the drug-loading efficacy and restrict any initial burst. After characterization of the microspheres in terms of encapsulation efficacy and particle size, we confirmed the *in vitro* drug release and *in vivo* pharmacokinetic properties of the microspheres. Finally, the optimized alendronate microspheres were injected intramuscularly into ovariectomized (OVX) rats at various doses, after which we evaluated their anti-osteoporotic efficacy compared to daily oral alendronate by analyzing bone properties and biological markers of bone turnover.

2. Materials and methods

2.1. Materials

Sodium alendronate was obtained from Gador S.A. (Buenos Aires, Argentina). Poly(lactic acid-co-glycolic acid) (PLGA; Resomer RG504H; mole ratio of lactic acid to glycolic acid = 50:50; molecular weight (MW) = 54,000) was purchased from Boehringer Ingelheim (Ingelheim, Germany). Hyaluronic acid (MW = 1,000,000) was obtained from Kibun Food Chemical Co., Ltd. (Tokyo, Japan). Pamidronate, chitosan, gelatin, polyvinyl alcohol, polyoxyethylene (20) sorbitan monooleate (Tween 80), polyoxyethylene (20) sorbitan monolaurate (Tween 20), and 9-fluorenylmethyl chloroformate (FMOC) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Solvents for microsphere preparation and high-performance liquid chromatography (HPLC) analysis were from Merck KGaA (Darmstadt, Germany) and Fisher Scientific (Pittsburgh, PA, USA).

2.2. Animals

New Zealand White rabbits (males, 2.0–2.2 kg) and Sprague Dawley rats (females, 250–280 g) were purchased from Orient Co., Ltd. (Gyunggi-do, Republic of Korea). The animals were acclimatized for 1 week in an animal facility under controlled conditions of temperature (23 ± 2 °C), relative humidity (55 ± 10%), and light (12/12 h light/dark, with no ultraviolet exposure). The animals had free access to a laboratory diet (Purina Co., St. Louis, MO, USA) and ion-sterilized tap water. All of the experiments were performed in accordance with the NIH guidelines for the Care and Use of Laboratory Animals, and the guidelines of Institutional Animal Care and Use Committee (IACUC) of Seoul National University Medical Research Center (Seoul, Republic of Korea).

2.3. Preparation of alendronate microspheres

In the present study, alendronate microspheres were fabricated by a s/w/o/w multiple emulsion technique. Briefly, an internal aqueous phase was obtained by dispersing 100 mg sodium alendronate in 500-μL water, hyaluronic acid (0.75% (w/v) in water), chitosan (0.75% (w/v) in water), or gelatin (0.75% (w/v) in water) with Tween 80 (20% (w/v) in water). To prepare a polymer solution, 30-g PLGA and 5-g sorbitan trioleate were dissolved in 100 g dichloromethane or a mixture of dichloromethane and acetone (7:3, v/v). Then, 1 mL internal aqueous solution was emulsified in 10-mL polymer solution with vigorous stirring. To form a s/w/o/w multiple emulsion, 10-mL s/w/o primary emulsion were slowly added to 2000-mL external continuous phase comprising ethyl acetate and 0.5% polyvinyl alcohol in water (1:99, w/w), and the mixture was further emulsified using a homogenizer at 5000 rpm for 5 min. After mild stirring for 30 min, organic solvents were removed by filtration, and the remaining product was washed with distilled water three times and vacuum-dried for 24 h to obtain alendronate microparticles. Table 1 shows the composition of the various microspheres.

2.4. Particle size analysis and scanning electron microscopy examination

The particle size was measured using a laser diffraction particle size analyzer (Mastersizer X, Malvern Instruments Ltd., UK). In

Table 1
Composition of alendronate-loaded microspheres.

Formulation code	PLGA type and concentration in the oil phase (%, w/v)	Polymer concentration in the internal aqueous phase (%, w/v)	Composition of solvent in the oil phase
ALD-RG504H	RG504H (30%, w/v)		Dichloromethane
ALD-HA-RG504H-MC100	RG504H (30%, w/v)	Hyaluronic acid (0.75%, w/v)	Dichloromethane
ALD-HA-RG504H-MC70	RG504H (30%, w/v)	Hyaluronic acid (0.75%, w/v)	Dichloromethane-acetone (7:3, v/v)
ALD-CHITO-RG504H-MC70	RG504H (30%, w/v)	Chitosan (0.75%, w/v)	Dichloromethane-acetone (7:3, v/v)
ALD-GELA-RG504H-MC70	RG504H (30%, w/v)	Gelatin (0.75%, w/v)	Dichloromethane-acetone (7:3, v/v)