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Estradiol-loaded PLGA nanoparticles for improving low bone mineral density of cancellous bone caused by osteoporosis: Application of enhanced charged nanoparticles with iontophoresis

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ABSTRACT

Postmenopausal osteoporosis among older women, which occurs by an ovarian hormone deficiency, is one of the major public health problems. 17 β-estradiol (E2) is used to prevent and treat this disease as a drug of hormone replacement therapy. In oral administration, E2 is significantly affected by first-pass hepatic metabolism, and high dose administration must be needed to obtain drug efficacy. Therefore, alternative administration route is needed, and we have focused on the transdermal drug delivery system. In this study, we have prepared E2-loaded poly(DL-lactide-co-glycolide) (PLGA) nanoparticles for osteoporosis by using a combination of an antisolvent diffusion method with preferential solvation. The average particle diameter of the nanoparticles was 110.0 ± 41.0 nm and the surface charge number density was 82 times higher than that of conventional E2-loaded PLGA nanoparticles. Therapeutic evaluation of E2-loaded PLGA nanoparticles was carried out using ovariectomized female rats. Therapeutic efficacy was evaluated to measure bone mineral density of cancellous bone using an X-ray CT system. When the E2-loaded PLGA nanoparticles were administrated once a week, bone mineral density was significantly higher than that of the non-treated group at 60 days after the start of treatment. Also, in the group administered this nanoparticle twice a week, the bone mineral density increased significantly at 45 days after the start of treatment. From these results, it was revealed that E2-loaded PLGA nanoparticles with iontophoresis were useful to recover bone mineral density of cancellous bone, and it was also suggested that they extend the dosing interval of E2.

1. Introduction

Osteoporosis is defined as a systemic skeletal disease characterized by low bone mass and microstructural deterioration of bone tissue, with a consequent increase in bone fragility and susceptibility to fracture [1]. Postmenopausal osteoporosis among older women is one of the major public health problems, which occurs by ovarian hormone deficiency [2,3], and especially in countries in which society is aging, prevention of the osteoporosis is important [4]. 17 β-estradiol (E2) is used to prevent and treat this disease as a drug of hormone replacement therapy (HRT). In oral administration, E2 is significantly affected by first-pass hepatic metabolism, and high dose administration must be needed to obtain drug efficacy [5]. Therefore, alternative administration route is needed to keep suitable drug level in blood. Transdermal administration is one of the useful administration routes. It can avoid the effect of first-pass hepatic metabolism, and deliver therapeutic agent as systemic or local administration for long period of time [6]. We have studied E2-loaded poly(DL-lactide-co-glycolide) (PLGA) nanoparticles using iontophoresis [7–9], which promotes skin permeability by using electropotential energy [10–12]. We have reported transdermal delivery of E2-loaded PLGA nanoparticles using iontophoresis for the treatment of osteoporosis. Ovariectomized female rats were used as an animal model of osteoporosis. Although the bone mineral density of cortical bone was increased by using nanoparticle system with iontophoresis, the significant difference had not been observed in cancellous bone [13]. We assumed that this result was due to the low surface charge number density of E2-loaded PLGA nanoparticles and E2 was not sufficiently permeated through the skin. In the treatment of osteoporosis, it is important to increase the bone mineral density of cancellous bone, since similar to post-

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menopausal bone loss, ovariectomy induces the greater loss of cancellous than cortical bone [14]. Recently, we have reported a combination of an antisolvent diffusion method with preferential solvation for nanoparticles including a hydrophobic drug. PLGA nanoparticles having high surface charge number density were prepared, and they were beneficial for the iontophoretic transdermal delivery of therapeutic agents [15]. We assumed that this technique was useful for improving bone mineral density of cancellous bone by improving skin permeability of E2-loaded PLGA nanoparticles.

The main aim of the present study was to improve bone mineral density of cancellous bone in an animal model for osteoporosis by applying iontophoresis and E2-loaded PLGA nanoparticles which were prepared using a combination of an antisolvent diffusion method with preferential solvation. We have measured electrophoretic mobility of the particles at nine different ionic strengths and analyzed them to evaluate the surface charge density of the particles. An animal model for osteoporosis was produced from female rat because its pathophysiological response of skeleton is similar to that of human [16]. To evaluate the therapeutic efficacy, we have measured bone mineral density of cancellous bone of femur by using computed tomography (CT).

2. Materials and methods

2.1. Materials

PLGA (Mw: 10,000, monomer composition of lactic acid/glycolic acid = 75/25), trehalose dihydrate ($C_{12}H_{22}O_{11}\cdot 2H_2O$, purity >97%), *N*-methyl-2-pyrrolidone (NMP), L-arginine (purity >98%), polyvinyl alcohol (PVA, degree of polymerization: 500), indomethacin ($C_{19}H_{16}ClNO_4$, purity >98%), dichloromethane (CH_2Cl_2, purity >99.5%), isopropyl myristate ($C_{17}H_{34}O_2$, purity >95%) were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). 17 β-estradiol (E2, $C_{18}H_{24}O_2$, purity >97%) was purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Methanol (CH_4O, JP, USP/NF, EP) and acetonitrile (CH_3CN, JP, USP/NF, EP) were purchased from Kanto Chemical Co., Inc. (Tokyo, Japan). Pentobarbital sodium salt ($C_{11}H_{17}N_2NaO_3$, purity >95%) was purchased from Nacalai Tesque, Inc. (Kyoto, Japan). Isoflurane for the animal was purchased from Mylan Inc. (Pittsburgh, Pennsylvania). Other chemicals were of the highest reagent grade commercially available.

2.2. Preparation of E2-loaded bare and PVA-coated PLGA nanoparticles

E2-loaded bare PLGA nanoparticles (bare nanoparticles) were prepared by using a combination of an antisolvent diffusion method with preferential solvation [15]. After 2 mg of E2 and 98 mg of PLGA were dissolved in a mixture of NMP (1 mL) and ethanol (1 mL), 10 μL of isopropyl myristate was added into the solution. The organic phase was rapidly added into 20 mL of 0.1% (w/v) L-arginine solution. Obtained nanoparticle suspension was ultracentrifuged at 40,000 rpm for 30 min (Himac 80WX, Hitachi Koki Co. Ltd., Tokyo, Japan). The precipitated nanoparticles were washed three times with distilled water to remove residual NMP. The obtained nanoparticles were centrifuged at 10,000 rpm for 3 min to remove aggregated nanoparticles and the supernatants were collected as bare nanoparticles suspension. Then trehalose dihydrate, which was used as a cryoprotectant, was added to the suspension until the ratio of nanoparticles to trehalose dihydrate was 1:2 [17]. After freezing at −30° C, this suspension was lyophilized by usage of a freeze dryer (FDU-1200, Tokyo Rikakikai Co., Ltd., Tokyo, Japan).

E2-loaded PLGA nanoparticles (PVA-coated nanoparticles) were also prepared by using emulsification and the solvent evaporation method to evaluate the effect of the preparation method on the physicochemical properties of nanoparticles. Ten milligrams of E2 and 490 mg of PLGA were dissolved in a mixture of 10 mL of dichloromethane and 10 mL of acetone. The solution was added to 100 mL of 1.0% (w/v) PVA aqueous solution, and was emulsified using a probe sonicator (Digital Sonifier S-250D, Branson Ultrasonics Co., Danbury, CT) at 100 W of energy output for 4 min on ice bath. Prepared O/W emulsion was stirred during 12 h on a magnetic stir plate at room temperature to evaporate the organic solvents. Obtained nanoparticle suspension was ultracentrifuged at 40,000 rpm for 30 min (Himac 80WX, Hitachi Koki Co. Ltd., Tokyo, Japan). The precipitated nanoparticles were washed with distilled water to remove residual PVA. The nanoparticles suspension was ultracentrifuged at 40,000 rpm for 20 min, and precipitated nanoparticles were washed two times with distilled water. The obtained nanoparticles were redispersed in distilled water. Then, it was centrifuged at 10,000 rpm for 10 min to remove aggregated nanoparticles, and the supernatants were collected as PVA-coated nanoparticles suspension. Trehalose dihydrate was added to the suspension until the ratio of nanoparticles to trehalose dihydrate was 1:1. After freezing at − 30° C, this suspension was lyophilized by usage of a freeze dryer (FDU-1200, Tokyo Rikakikai Co., Ltd., Tokyo, Japan).

2.3. Physicochemical evaluation of E2-loaded PLGA nanoparticles

The morphology of nanoparticles was observed using scanning electron microscope (SEM, JSM-6060LA, JEOL Ltd., Akishima, Japan). The mean volume diameter, the size distribution and the electrophoretic mobility of bare nanoparticles and PVA-coated nanoparticles were measured using a zeta-potential and particle size analyzer (ELSZ-2, Otsuka Electronics Co., Ltd., Osaka, Japan). The electrophoretic mobility of the nanoparticles were measured in physiological saline with nine different ionic strengths (I = 0.005, 0.010, 0.020, 0.040, 0.060, 0.075, 0.100, 0.125, 0.154 M), at skin surface temperature (32° C) [18]. The loading ratio of E2 in the prepared nanoparticles was measured using a high-performance liquid chromatography (HPLC) system at 281 nm. The HPLC system consists of an UV detector (SPD-20A prominence, SHIMADZU Co., Kyoto, Japan), a pomp (LC-20AD prominence, SHIMADZU Co., Kyoto, Japan), an auto-sampler (SIL-20A, SHIMADZU Co., Kyoto, Japan), a column oven (CTO-10ASvp, SHIMADZU Co., Kyoto, Japan), a degassing apparatus (DGU-20A_3 prominence, SHIMADZU Co., Kyoto, Japan), and an ODS column (Develosil ODS-HG-5, size: 4.6 × 150 mm, Nomura Chemical Co. Ltd., Seto, Japan). To prepare mobile phase, 0.1 M of acetic acid solution was mixed with an equivalent amount of acetonitrile. Indomethacin solution (0.2 μg/mL), made of indomethacin powder and mobile phase, was used as an internal standard substance solution. Fifteen milligrams of prepared nanoparticles were dissolved in 4 mL of the mobile phase, and 0.1 mL of internal standard substance solution were added to 1.9 mL of this solution. HPLC measurement was carried out at 40° C (flow rate of 1.5 mL/min) and 80 μL of the sample solution was applied. All HPLC measurements were carried out under the same conditions.

To evaluate the stability of E2-loaded PLGA nanoparticles in donor phase of ex vivo and in vivo permeability studies, release rates of E2 from the nanoparticles were studied. In brief, bare nanoparticles or PVA-coated nanoparticles were redispersed in 5 mL of physiological saline (0.005 M), and suspension including 0.1 mg/mL E2 were prepared. The suspensions were shaken at 30 rpm at 32° C. After 1, 2, 4, 6, and 8 h, the samples were taken and centrifuged at 40,000 rpm for 30 min. Then, 0.5 mL of the supernatants were collected and added to 1.4 mL of mobile phase with 0.1 mL of the internal standard solution. For determining released E2 amount, HPLC measurement was carried out. We have also measured release rates of E2 from the nanoparticles at 37° C to evaluate