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Osteotropic β-cyclodextrin for local bone regeneration

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Abstract

An osteotropic alendronate-β-cyclodextrin conjugate (ALN-β-CD) was developed as a bone-targeting delivery system for improved treatment of skeletal diseases. The conjugate shows very strong binding to hydroxyapatite (HA, main component of the skeleton). Its ability in forming molecular inclusion complex with prostaglandin E_1 (PGE_1, a potent bone anabolic agent) was confirmed by phase solubility experiments and differential scanning calorimetry (DSC). In a bilateral rat mandible model, ALN-β-CD/PGE_1 molecular complex was shown to stimulate strong local bone anabolic reaction. In the control study, ALN-β-CD itself was also found to be bone anabolic. To investigate this finding, other control groups were studied. The histomorphometry data suggest that ALN-β-CD itself could generate more new bone at the injection site than its complex with PGE_1. Alendronate (ALN) injection could also cause new bone formation, which locates peripheral to the site of injection. PGE_1, saline or ethanol injections do not have anabolic effect. These findings were also confirmed by micro-CT evaluation of mandibular bones. It is clear that the bone anabolic effect of ALN-β-CD is independent of mechanical stimuli of the periosteum or ALN injection alone. Further studies are warranted to understand the working mechanism of ALN-β-CD as a bone anabolic agent.

Keywords: Bone regeneration; Osteoporosis; Hydroxyapatite; Drug delivery

1. Introduction

Repair of bone defects due to trauma, arthritis, cancer treatment or other skeletal diseases is expensive and invasive. Substantial research efforts in this area have been focused on effective incorporation of synergistic growth factors, such as bone morphogenetic proteins (BMPs), into the bone grafts [1–3]. Compared to these biologics, the clinical applications of low molecular weight bone anabolic agents such as prostaglandins (E_1 and E_2) [4,5], statins [6] and prostaglandin EP_4 receptor agonists [7,8] have not yet been developed. Prostaglandins are locally secreted, rapidly metabolized, biologically active fatty acids first identified in the prostate [9]. The potency of PGEs as authentic bone anabolic agents was demonstrated convincingly in vivo by both systemic and local delivery [10]. However, the clinical applications of these agents have been limited by their profound side effects on the soft tissues. Yet their outstanding anabolic potency, low cost and relatively stable chemical structures are intriguing for clinical bone anabolic therapy development. With the applications of appropriate delivery systems, one may anticipate widespread clinical applications of the PGEs to accelerate bone defect repair.

Cyclodextrins (CDs) are water-soluble cyclic oligosaccharides, composed of α-D-glucopyranoside units linked 1–4. The naturally occurring CDs are α, β and γ CDs having 6, 7 and 8 glucopyranose units, respectively. The annulus interior

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Abbreviations: ALN, alendronate; ALN-β-CD, alendronate-β-cyclodextrin conjugate; β-CD, β-cyclodextrin; DSC, differential scanning calorimetry; EDC, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride; HA, hydroxyapatite; HP-β-CD, 2-hydroxylpropyl-β-cyclodextrin; NHS, *N*-hydroxysuccinimide; PGE1, prostaglandin E1; RB, rhodamine B; RB-β-CD, RB-labeled β-CD; RB-ALN-β-CD, RB-labeled ALN-β-CD.

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of CD is hydrophobic, which enables the formation of inclusion complexes with many lipophilic compounds. Being considered biologically inert, CDs have been approved by US FDA as pharmaceutical excipients for numerous drug formulations [11]. Recently, we have designed and synthesized an alendronate-β-cyclodextrin (ALN-β-CD) conjugate for local delivery of therapeutic agents to the bone and teeth [12]. In this manuscript, this conjugate was studied as a delivery system for prostaglandin E_1 for the treatment of bone defects. Surprisingly, when evaluated in vivo, the new β-CD derivative showed a robust bone anabolic effect.

2. Materials and methods

2.1. Materials

β-CD was purchased from TCI America (Portland, OR, USA). *p*-Toluenesulfonyl chloride, 4-pentynoic acid, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), *N*-hydroxysuccinimide (NHS), sodium azide, $CuSO_4 \cdot 5H_2O$, sodium ascorbate, dimethylformamide and dichloromethane were purchased from Acros Organics (Morris Plains, NJ, USA). Alendronate (ALN) was purchased from Ultratech India Ltd. (New Mumbai, India). Hydroxyapatite (HA, DNA grade Bio-Gel HTP gel, surface area ≈ 50 m^2/g) was purchased from Bio-Rad (Hercules, CA, USA). Prostaglandin E_1 (PGE_1) was purchased from Hawkins Inc. (Minneapolis, MN, USA). The Sprague-Dawley rats (retired breeder) were purchased from Charles River Laboratories, Inc. (Wilmington, MA). All other reagents and solvents if not specified were purchased from Fisher Scientifics (Pittsburgh, PA, USA).

2.2. Synthesis of ALN-β-CD

ALN-β-CD (Fig. 1) was synthesized according to the method we reported previously [12]. Briefly, β-cyclodextrin was first toslated with *p*-toluenesulfonyl chloride and then converted to mono-6-(azido)-β-cyclodextrin (N_3-β-CD). Active ester of 4-pentynoic acid was synthesized by 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) coupling 4-pentynoic acid with *N*-hydroxysuccinimide (NHS), then it reacted with alendronate in water under controlled pH to obtain 1-hydroxy-4-pent-4-ynamidobutane-1,1-diyldiphosphonic acid (alkyene-ALN). Finally, alkyene-ALN was conjugated to N_3-β-CD via Huisgen 1,3-dipolar cycloaddition (a "click" reaction) with $CuSO_4$/sodium ascorbate as catalysts to obtain ALN-β-CD.

2.3. Biomineral-binding ability of ALN-β-CD

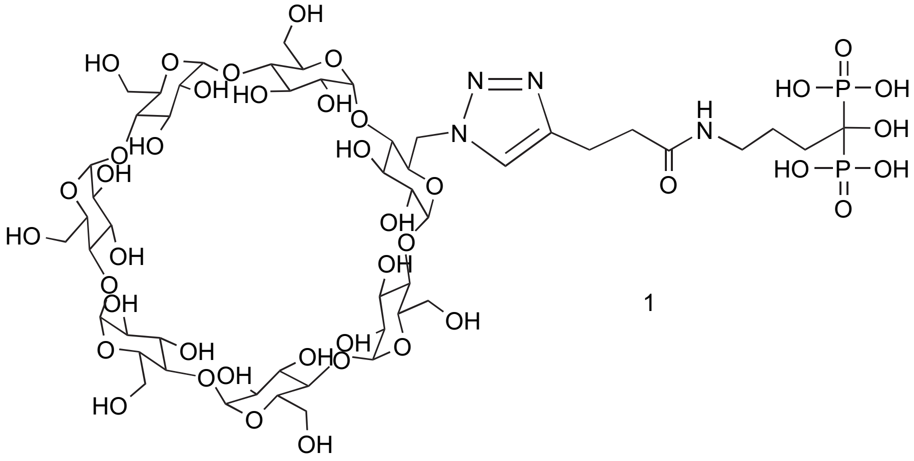
ALN-β-CD was first labeled with rhodamine B (RB) by EDC coupling. RB-ALN-β-CD (20 mg) was then dissolved in 0.5 mL H_2O and filtered through 0.22 μm filters. It was then agitated with HA (100 mg) for 10 min at 21 °C, HA was removed by centrifugation (10,000 rpm, 2 min), and then washed extensively with H_2O and acetone. The recovered HA was dried under vacuum. RB-labeled β-CD (RB-β-CD) and RB were used as controls for non-specific binding.

2.4. Saturated binding of ALN-β-CD on HA

ALN-β-CD (100 mg) and RB-ALN-β-CD (0.2 mg) were dissolved together in PBS (5 mL, pH = 7.4, 10 mM) and filtered through 0.22 μm filters. HA powder (50 mg) was added into 1 mL of this solution. After 10 min of agitation at 21 °C, HA was removed by centrifugation (10,000 rpm, 2 min). The absorbance of the supernatant at 560 nm was determined using a UV/VIS spectrophotometer (UV-1601PC, Shimadzu, Kyoto, Japan) and compared with that of the initial RB-ALN-β-CD solution. The analysis was performed in triplicate.

2.5. Phase solubility study of PGE_1 in the presence of ALN-β-CD

The solubility study was performed according to Higuchi and Connors [13]. An excess amount of PGE_1 (2 mg) was added to aqueous solutions (1.0 mL, pH



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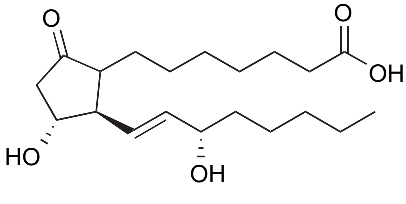

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Fig. 1. The chemical structures of ALN-β-CD (1) and PGE_1 (2).