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Enteric-coated tablet of risedronate sodium in combination with phytic acid, a natural chelating agent, for improved oral bioavailability

Jeong S. Kim [a], Sun W. Jang [a], Miwon Son [a], Byoung M. Kim [a], Myung J. Kang [b,*]

[a] Dong-A Pharmaceutical Co. Ltd., Giheung-gu, Yongin, Gyeonggi, 446-905, Republic of Korea
[b] College of Pharmacy, Dankook University, 119 Dandae-ro, Dongnam-gu, Cheonan, Chungnam 330-714, Republic of Korea

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ABSTRACT

The oral bioavailability (BA) of risedronate sodium (RS), an antiresorptive agent, is less than 1% due to its low membrane permeability as well as the formation of non-absorbable complexes with multivalent cations such as calcium ion (Ca^{2+}) in the gastrointestinal tract. In the present study, to increase oral BA of the bisphosphonate, a novel enteric-coated tablet (ECT) dosage form of RS in combination with phytic acid (IP6), a natural chelating agent recognized as safe, was formulated. The chelating behavior of IP6 against Ca^{2+}, including a stability constant for complex formulation was characterized using the continuous variation method. Subsequently, in vitro dissolution profile and in vivo pharmacokinetic profile of the novel ECT were evaluated comparatively with that of the marketed product (Altevia, Sanofi, US), an ECT containing ethylenediaminetetraacetic acid (EDTA) as a chelating agent, in beagle dogs. The logarithm of stability constant for Ca^{2+}–IP6 complex, an equilibrium constant approximating the strength of the interaction between two chemicals to form complex, was 19.05, which was 3.9-fold ($p < 0.05$) and 1.7-fold ($p < 0.05$) higher than those of Ca^{2+}–RS and Ca^{2+}–EDTA complexes. The release profile of RS from both enteric-coated dosage forms was equivalent, regardless of the type of chelating agent. An in vivo absorption study in beagle dogs revealed that the maximum plasma concentration and area under the curve of RS after oral administration of IP6-containing ECT were approximately 7.9- ($p < 0.05$) and 5.0-fold ($p < 0.05$) higher than those of the marketed product at the same dose (35 mg as RS). Therefore, our study demonstrates the potential usefulness of the ECT system in combination with IP6 for an oral therapy with the bisphosphonate for improved BA.

1. Introduction

Risedronate sodium (RS), a potent pyridinyl bisphosphonate, has been frequently prescribed as the first-line therapeutic agent for the treatment of Paget's disease, osteoporosis, and other bone disorders (Pazianas et al., 2010; Cremers and Papapoulos, 2011). The bisphosphonate has a high affinity for bone minerals including calcium (Ca^{2+}) and magnesium ions (Mg^{2+}), and thus binds strongly to the surfaces of skeletal structure. The ingestion of the compound by osteoclasts in the bisphosphonate-bound bone leads to their death via apoptosis, preventing systemic bone resorption in osteoporosis or other bone diseases (Russell et al., 2008; Iolascon et al., 2010). Of the commercially available bisphosphonates, the nitrogen-containing-compounds such as pamidronate, alendronate, ibandronate and RS exhibit 100–10,000-fold higher antiresorptive activity compared to non-nitrogen-containing-bisphosphonates including etidronate (Russell et al., 1999). However, one of the major problems in the clinical use of RS is its extremely poor and variable oral absorption, with the bioavailability (BA) in healthy subjects being less than 1% (Mitchell et al., 2000). The high hydrophilicity and extensive ionization of the compound in the gastrointestinal tract prevent transcellular permeation across the epithelial membrane. Moreover, the presence of Ca^{2+} or other divalent cations in the intestinal lumen hampers its absorption, by forming non-absorbable complexes with the metal ions (Lambrinoudaki et al., 2006; Kinov and Boyanov, 2012).

To improve the oral BA, the originator formulated a once-weekly regimen for RS by employing an enteric-coated tablet (ECT) containing ethylenediaminetetraacetic acid (EDTA) as chelating agent against multivalent cations (Atelvia, Sanofi, US). The commercialized enteric-coated dosage form contains 35 mg anhydrous RS in the form of hemi-pentahydrate with 100 mg EDTA. The enteric-coated dosage form is intended to carry the active compound and EDTA to the small intestine where the mineral concentrations are relatively lower than the stomach, and subsequently, the chelating agent competitively forms complexes with metal ions such as Ca^{2+} instead of the bisphosphonate, preventing growth of non-absorbable precipitates with metal ions

* Corresponding author at: College of Pharmacy, Dankook University, 119 Dandae-ro, Dongnam-gu, Cheonan, 330-714, Republic of Korea.
E-mail address: kangmj@dankook.ac.kr (M.J. Kang).

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(Pazianas et al., 2013). When Atelvia was taken after an overnight fast and followed by a 4-hour fast, the oral absorption of RS was approximately 44% higher than that of the immediate-release tablet in healthy subjects (Atelvia, 2010). Unfortunately, however, the marketed product contained a large amount of EDTA, causing a high risk of abdominal pain, and thus, should be taken immediately following breakfast and not under fasting conditions. The post-prandial administration of the marketed product remarkably decreased the oral absorption of RS by 30% in healthy subjects (Atelvia, 2010). Moreover, 100 mg of EDTA contained in the marketed product is a considerable quantity, reaching more than two-thirds of the recommended acceptable daily intake dose (2.5 mg/kg) (Galán et al., 2012). Therefore, there is an unmet need for the development of an alternative formulation for RS with less gastrointestinal adverse effects and a better oral BA.

Phytic acid, also known as *myo*-inositol hexaphosphate (IP6, Fig. 1), is an organic acid extracted from rice bran (Graf et al., 1987). The natural compound has been reported to possess various health benefits as an antioxidant, anticancer agent, and chelating agent (Kunyanga et al., 2011; Shamsuddin et al., 1997; Jariwalla, 2001). Structurally, the organic acid contains six phosphates and has a strong chelating action against diverse metal cations such as Mg^{2+} and Zn^{2+}. IP6 has a chelating stability constant (β) against Mg^{2+} or Zn^{2+} that is quite comparable with those of EDTA (Sato, 1986). Previous studies have revealed that IP6 noticeably facilitated intestinal absorption of coadministered therapeutic agents such as anthocyanins and several flavonoids in rats and/or humans, although the exact mechanisms are unclear (Matsumoto et al., 2007; Xie et al., 2014). Moreover, the natural compound has been approved as a generally recognized safe (GRAS) material by the Food and Drug Administration (FDA) in the US. A previous toxicity study of IP6 on rat intestine evidenced that oral intake of the natural compound up to a dose of 200 mg/kg did not result in any irritation to the duodenum, jejunum, ileum, or colon segments (Xie et al., 2014).

Therefore, the objectives of the present study are to formulate an ECT dosage form of RS in combination with IP6, and to investigate the influence of IP6 on oral BA of the bisphosphonate. At first, the chelating capability of IP6 with Ca^{2+} was estimated by determining the logarithm of β (logβ) of Ca^{2+}:IP6 complex using a continuous variation method, and was compared with those of the bisphosphonate and EDTA, respectively. We subsequently compressed the IP6-containing ECT using conventional wet granulation and compaction, followed by an enteric coating process. The in vitro dissolution profile and in vivo pharmacokinetic profile of RS following oral administration of the novel dosage form were comparatively evaluated with those of the marketed product (Altevia, Sanofi, US) in beagle dogs.

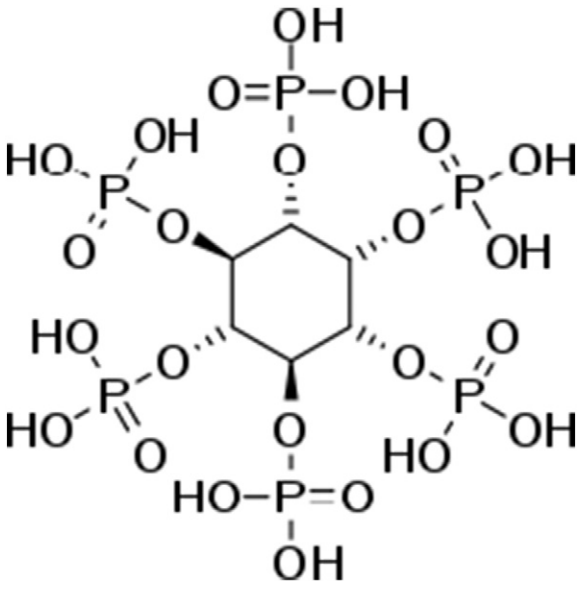

Fig. 1. Chemical structure of IP6, a naturally occurring chelating agent.

2. Materials and methods

2.1. Materials

The RS powder was obtained as a gift sample from Teva Pharmaceuticals (Petah Tikva, Israel). RS-d4 was purchased from Toronto Research Chemicals, (North York, Ontario, Canada) as an internal standard for LC/MS–MS analysis. Sodium EDTA, calcium chloride, and trimethylsilyl diazomethane were purchased from Sigma Chemical Co. (St. Louis, MO, USA). IP6 solution (80%) was obtained from Tsuno Food Industrial Co. (Wakayama, Japan). Microcrystalline cellulose (Avicel® PH 112) and Acryl-EZE® (Eudragit L100-55-based aqueous acrylic enteric system) were kindly provided by FMC Corporation (Philadelphia, USA) and Colorcon Korea (Gyunggi-do, Korea), respectively. Sodium starch glycolate (Primojel®) was purchased from DFE Pharma (Nörten-Hardenberg, Germany). Magnesium stearate, ammonium bicarbonate, potassium hydroxide, and formic acid were obtained from Duksan Co Ltd. (Seoul, Korea). All other chemicals were of reagent grade and used without further purification.

2.2. Determination of the logβ of RS, IP6, and EDTA against Ca^{2+}

2.2.1. Determination of the logβ of Ca^{2+}:RS and Ca^{2+}:IP6 complexes

A logβ, an equilibrium constant for the formation of a complex in medium, has been commonly used to estimate the strength of the interaction between ligands and ions that form coordination compounds in solution. The chelating behaviors of the bisphosphonate and IP6 against Ca^{2+}, including stoichiometric ratio of the ligand to the metal ion and their logβ values, were determined using a continuous variation method as previously reported with slight modifications (Takenaka et al., 2005; Renny et al., 2013). In brief, calcium chloride, RS, and IP6 solutions were prepared in 1.0 M hexamine buffer (pH 6.0) at a concentration of 4 mM. A set of samples was prepared by mixing Ca^{2+} solution with the drug or IP6 solution in different ratios from 1:9 to 9:1. The samples were placed in a water bath and shaken (60 rpm) at 37 ± 0.1 °C for 24 h, which had been previously determined to be an adequate time for equilibration. Each sample was then filtered using a 0.45 μm PVDF syringe filter to remove insoluble Ca^{2+}:ligand complexes. The filtrate was diluted with distilled water, and the concentrations of free RS and IP6 in the solution were determined by HPLC. Then β value of the complexes was calculated as follows:

$$\beta = \frac{[M_nA]}{[M]^n[A]}$$

where, $[M_nA]$ is the molar concentration of the complex, $[M]$ is the molar concentration of the free metal ion, $[A]$: the concentration of the uncomplexed RS or IP6, and n is the number of moles of metal ion chelated with one mole of ligand.

Chromatographic analysis for RS was carried out using a Dionex-Ultimate 3000 HPLC system equipped with an LPG-3400SD pump, a WPS-3000SL auto-sampler, a TCC-3000 column oven, and a DAD-3000 UV–VIS diode array detector. An anion-exchange column (IonPac® AG7: 10 μm, 50 mm × 4.0 mm, Dionex) was used with an EDTA solution (1.8 mg/ml, pH 9.5) as a mobile phase. The effluent was monitored at the UV absorption wavelength of 263 nm at the flow rate of 0.8 ml/min. Free IP6 in the medium was determined by an ion-pair reversed-phase HPLC assay. A C18 column (Spherisor ODS, 5 μm, 250 mm × 4.0 mm), maintained to 45 °C, was run with a mobile phase consisting of methanol:water:tetrabutyl ammonium (51:48:1, pH 4.3). The effluent was detected by refractive index with a flow rate of 1.2 ml/min.