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Gastroresistant microparticles containing sodium alendronate prevent the bone loss in ovariectomized rats

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ABSTRACT

Sodium alendronate, an antiresorptive drug, primarily used in the treatment of osteoporosis was encapsulated in blended microparticles composed of Eudragit® S100 and Methocel® K15M. The micropowder obtained by spray-drying technique was characterized in terms of its morphology, particle size, encapsulation efficiency and *in vitro* drug release. *In vivo* studies were carried out in order to evaluate the pharmacodynamic effect and the ulcerogenic activity of sodium alendronate-loaded microparticles after oral administration in rats. Drug encapsulation efficiency was close to 80% and particle mean diameter of 13.8 μm. SEM analysis showed spherical collapsed shape particles with smooth surface. At pH 1.2, *in vitro* experiments showed that <10% of the drug was released from the microparticles. At pH 6.8, the microparticles were able to prolong the sodium alendronate release for 12 h. *In vivo* experiments carried out in ovariectomized rats showed bone mineral density significantly higher for the sodium alendronate-loaded microparticles than for the negative control groups. Furthermore, the microencapsulation of the drug showed a significant reduction in the ulcerative lesion index. In conclusion, the blended microparticles are excellent oral carriers for sodium alendronate since they were able to maintain the drug antiresorptive effect and to reduce the gastrointestinal drug toxicity.

1. Introduction

Osteoporosis is a progressive systemic disease associated with a diminution of bone mass and a deterioration of the microarchitecture, causing bone fragility and increasing the risk of fractures (Mundy, 2000; Pérez-Lopez, 2004). This disorder is characterized by an imbalance in bone remodeling in which the rate of bone resorption exceeds that of bone formation (Pérez-Lopez, 2004). Bisphosphonates suppress bone resorption and bone turnover by their affinity for hydroxyapatite crystals of bone and selective inhibition of osteoclastic bone resorption during the remodeling cycle (Pérez-Lopez, 2004; Stepan et al., 2003).

Sodium alendronate, an aminobisphosphonate, has demonstrated in controlled trials progressive increases in bone mineral density at the lumbar spine and proximal femur and a significant reduction in vertebral, hip, and wrist fractures of postmenopausal women with osteoporosis (Devogelaer et al., 1996; Felsenberg et al., 1998). In chronic diseases, such as osteoporosis, oral dosage forms are the most common and convenient for patients. Currently, sodium alendronate is available as an oral daily regimen or as single tablet taken once a week. However, sodium alendronate oral treatment has been associated with damage in upper gastrointestinal mucosa (Aki et al., 2003). Due to low bioavailability and the potential for gastrointestinal irritation, bisphosphonates must be taken following an overnight fast with a glass of water, at least 30 min before ingesting food, drink or medication. Patients are also required to remain sitting or standing upright for 30 min post-dose (Li and Kendler, 2004). To follow such a routine is inconvenient. However, to not comply with these instructions may lead to gastrointestinal discomfort, one of the reasons cited for patients discontinuing bisphosphonate therapy (Tosteson et al., 2003). In this context, polymeric microparticles represent an interesting alternative as carriers for oral delivery of sodium alendronate.

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Microparticulate carriers have been developed to modify the pharmacokinetic profiles of drugs (Palmieri et al., 2000; Freiberg and Zhu, 2004; Kurkuri and Aminabhavi, 2004), to protect unstable molecules from degradation (Esposito et al., 2002; Desai and Park, 2005; Colomé et al., 2007; Raffin et al., 2008) and to reduce the drug toxicity, mainly in the gastrointestinal tract (Castelli et al., 1998; Palmieri et al., 2000).

Eudragit® S100, used to prepare microparticles, is an anionic polymer that presents pH-dependent solubility. It is insoluble in low pH and soluble in the region of the digestive tract where juices are neutral to weakly alkaline (Ammar and Khalil, 1997). So, this polymer is mainly used to achieve gastroresistant microparticulate carriers (Raffin et al., 2008). Hydroxypropyl methylcellulose (HPMC; Methocel brand), a non-ionic cellulose ether derivative, is a hydrophilic swellable polymer frequently employed to provide drug controlled release (Li et al., 2005). Previous experiences in our group showed that enteric and controlled release microparticles were successfully prepared using a blend of Eudragit® S100 and HPMC to protect pantoprazole against acid degradation in the stomach lumen providing intestinal release of the drug (Raffin et al., 2006, 2007, 2008). More recently, we have reported that sodium alendronate was successfully entrapped in blended microparticles prepared by spray-drying with Eudragit® S100 and two different HPMC viscosity grades (Methocel® K100LV or Methocel® F4M). In vitro dissolution studies showed good gastroresistance and retarded release for both formulations (Cruz et al., 2009). So, with the objective to verify if those blended microparticles were able to maintain the pharmacodynamic effect and to increase the gastrointestinal tolerance of the drug, the purpose of this study was to evaluate the antiresorptive activity and the ulcerative effect of sodium alendronate-loaded blended microparticles in the prevention of osteoporosis in ovariectomized rats. Hence, a new sodium alendronate formulation was prepared using blended microparticles composed by Eudragit® S100 and Methocel® K15M. Eudragit® S100 was chosen to entrap sodium alendronate viewing to protect upper gastrointestinal mucosa from irritant effects of the drug, and Methocel® K15M was selected to increase the microgel viscosity and ensure a drug sustained release.

2. Materials and methods

2.1. Materials

Monosodium alendronate trihydrate was purchased from Henrifarma (São Paulo, Brazil). Eudragit® S100 was obtained from Almapal (São Paulo, Brazil) and Methocel® K15M was supplied by Colorcon (Cotia, Brazil). *o*-Phthalaldehyde (OPA) was obtained from Invitrogen (Carlsbad, USA) and 2-mercaptoethanol was acquired from Acros Organics (Geel, Belgium). All other chemicals and solvents were of pharmaceutical grade and used as received.

2.2. Preparation of sodium alendronate-loaded microparticles

Microparticles (MP) were prepared by the spray-drying technique. Eudragit® S100 (5.0 g) was dissolved in 0.05 mol L^{-1} NaOH (500 mL) under magnetic stirring at 50 °C. After dissolution, HPMC (2.5 g) was added and the mixture was kept under mechanic stirring for 10 min. The formed gel was kept at 4 °C for 48 h until the complete dissolution of HPMC. Sodium alendronate (2.0 g) was added in the gel before spray-drying. The operational conditions were: feed rate of 0.40 L h^{-1}, air flow rate of 500 N L h^{-1}, atomizing air pressure of 3.7 kgf $(cm^2)^{-1}$, inlet temperature of 150 °C and nozzle diameter of 1.2 mm. Microparticles prepared without the drug (placebo microparticles) were used as control. All formulations were prepared in triplicate.

2.3. Physicochemical characterization of microparticles

The yield of the drying process was calculated by the ratio of the experimental weight of the powder and the sum of the solid components weights. Concerning the encapsulation efficiency, a spectrophotometric validated method based on the derivatization of sodium alendronate with *o*-phthalaldehyde was used (Al Deeb et al., 2004; Cruz et al., 2009). An amount of microparticles (52.5 mg) equivalent to 10 mg of sodium alendronate was dissolved in 50 mL of 0.2 mol L^{-1} NaOH and filtered using a filter paper. Ten milliliters of the filtrate were transferred to another 50 mL volumetric flask. Then, 4 mL of derivatizing reagent were added and the volume was completed with 0.2 mol L^{-1} NaOH. After 30 min, the absorbance was measured at 333 nm (Cary 50 UV–Vis, Varian, USA). The encapsulation efficiency of each formulation was calculated based on the relation of the theoretical and the experimental sodium alendronate concentrations, and expressed as percentage. Each sample was assayed in triplicate.

Size and size distributions of microparticles were analyzed by laser diffractometry (Malvern Mastersizer, 2000, Malvern Instruments, UK) after dispersion of microparticles in *iso*-octane. The particle sizes were expressed as the mean diameter over the volume distribution $d_{4.3}$ and the size distributions (*span*) were calculated using Eq. (1):

$$span = \frac{[d(0.9) - d(0.1)]}{d(0.5)} \quad (1)$$

where $d_{0.1}$, $d_{0.5}$ and $d_{0.9}$ are, respectively, the particle diameters at 10%, 50% and 90% of the undersized particle distribution curve.

Shape and surface of the microparticles were examined by means of a scanning electron microscopy (SEM) (Jeol Scanning Microscope, JSM-5800, Tokyo, Japan). The powders were carbon and gold sputtered (Jeol Jee 4B SVG-IN, Tokyo, Japan) before analyses (Centro de Microscopia Eletrônica-UFRGS, Porto Alegre, Brazil) (Lionzo et al., 2007).

2.4. In vitro drug release studies

The *in vitro* drug release experiments were performed in a dissolution apparatus (Vankel VK7010, VanKel, USA) at 37 °C, using the basket method at a rotation speed of 100 rpm. To evaluate the gastroresistance, the microparticles were poured into a vessel containing 900 mL of 0.1 mol L^{-1} HCl (pH 1.2). Samples were withdrawn every half-hour up to 2 h and analyzed spectrophotometrically using OPA as derivatizing agent. According to The United States Pharmacopeia, to evaluate the gastroresistance of a dosage form, formulations should be assayed in gastric simulated fluid pH 1.2 (prepared with 0.1 mol L^{-1} HCl) for 2 h because is the time of gastric emptying. The samples (1 mL) were treated with OPA (0.4 mL) and the volume was volumetrically completed to 5 mL with 5 mol L^{-1} NaOH instead of 0.2 mol L^{-1} NaOH aqueous solution used to determine the encapsulation efficiency in order to obtain sodium alendronate conversion for the highest sensitivity once the derivatization of sodium alendronate with OPA is a reaction pH-dependent with optimum pH at 12.7. Absorbances were measured at 333 nm after 30 min.

To evaluate the sodium alendronate release profiles, the dissolution tests were performed in 900 mL phosphate buffer pH 6.8. The samples were collected at predetermined intervals (10, 15, 30, 45, 60, 90, 120, 150, 180, 240, 300, 360, 420, 480, 540, 600, 660 and 720 min). The amount of sodium alendronate released was analyzed by the derivatization of the samples (1 mL) with OPA (0.4 mL) in a volumetric flask (5 mL). The volume was completed with 0.2 mol L^{-1} NaOH. After 30 min, the absorbances were analyzed at 333 nm. For both media, the dissolution of the pure