ORIGINAL PAPER

IN VITRO RELEASE STUDIES OF ALENDRONATE FROM HA-AL COMPOSITE DEPOSITED ON TITANIUM METAL SUBSTRATE

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Manuscript received: 22.03.2019; Accepted paper: 16.06.2019; Published online: 30.06.2019.

Abstract. *Bisphosphonates are medicines used to inhibit osteoclastic activity in a number of bone diseases (Paget's disease, osteoporosis, hypercalcemia, primary and secondary hyperparathyroidism, bone metastases). Following in vitro release studies, crystalline hydroxyapatite (HA) was found to be a stable phase in contact with the release medium, and alendronate (AL) bound to HA by the synthesis process was slowly released from the metallic component (titanium) into the release medium for 10 days. Hydroxyapatite after implantation produces chemical species that support the adhesion of the implant to the surrounding tissue forming a functional connective structure. Thus synthesis of the HA-AL composite can be considered as a viable solution for the inclusion of bisphosphonate on the surface of metallic prosthetic components used in orthopedics.*

Keywords: *bisphosphonates, hydroxyapatite- alendronate, implant.*

1. INTRODUCTION

Bone disorders are difficult to treat because the blood flow that reaches the bone level is low and implicitly the drug concentration is reduced. If there is an incompatibility between metallic implants and bone structure, it is favored to develop an inflammatory response as a way of defending the body who treat prosthesis as a foreign body. That's why metal implants need to be compatible with the bone structure.

Recently, special attention has been focused among researchers to obtain biomaterials for the purpose of bone tissue reconstruction. Among these biomaterials, hydroxyapatite has a number of outstanding properties, such as biocompatibility and bioactivity, which is commonly used to make bone grafts and to cover metallic components of prostheses used in orthopedics.

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Even though HA has been available as synthetic material for over 15 years and has been used in ceramic implantology for more than a decade, new solutions are being sought to include certain anionic or cationic substituents in its structure to bring its composition close to biological apatite.

The total hip or knee arthroplasty, cemented or non-cemented is a standard orthopedic surgical procedure that is used to relieve pain and improve the quality of life in arthritis patients, with satisfactory short and long-term results. The success of these interventions depends on the rapid fixation of the prosthesis components in the bone mass located on its surface.

The mobile components of this prosthesis generate small particles, "scraps" that stimulate monocytes and the phagocytic system. These cells attempt to eliminate coarse particles and thus create pro-inflammatory mediators and proteolytic enzymes that form a granuloma around the implant and cause a series of biochemical reactions that lead to bone resorption and osteolysis [1, 2].

Since the viability of the implant depends on the processes occurring at the bone-implant interface, the physico-chemical optimization of the implants surface used in orthopedic surgery is fundamental for achieving a consistent and rapid bone integration [3].

There is a great interest among physicists, biologists, and physicians for the development of biomimetic surfaces of calcium and protein phosphates that would improve cell adhesion and thus reduce bone integration time [4].

To avoid perprotetic bone loss, drug antiresorption therapy can be implemented by including bisphosphonates that inhibit osteoclast activity [5]. Bisphosphonates are used in many diseases such as Paget's disease of the bone, osteoporosis, hypercalcemia [6, 7].

Although the main effect of bisphosphonates is the inhibition of bone resorption of osteoclasts, there are studies showing a positive effect on osteoblasts. Thus, many studies show differential growth of progenitor cells of osteoblasts, with positive effects on their proliferation and maturation. At the same time, these studies demonstrate that bisphosphonates can prevent osteoblast apoptosis [8].

To avoid loss of bone mass and some side effects that may occur after long-term treatment (osteonecrosis), local bisphosphonates should be used. In this way, a high dose of drug can be administered in the area of interest in order to reduce bone loss with positive effects on bone integration time and implant fixation. Clinical studies after implantation have shown that systemic bisphosphonate administration results in a decrease in prostatic bone loss after only 3 months of treatment.

2. MATERIALS AND METHODS

2.1. MATERIALS

All chemicals were analytical reagent (AR) grade: $Ca(NO_3)_2 \cdot 4H_2O$ (Sigma-Aldrich, ≥99%); $(NH_4)_2HPO_4$ (Sigma-Aldrich, ≥99%); NH_3 (Sigma- Aldrich, 30 - 33%); alendronate sodium salt (Axxora, ≥ 97%); HPLC Water (LiChrosolv® Merck); FMOC, acetonitrile, sodium citrate solution (Sigma-Aldrich).

2.2. METHODS

***Synthesis of hydroxyapatite-alendronate*:** In order to obtain the composite were prepared two solutions of calcium nitrate tetrahydrate of 1.08 M and 0.65 M ammonium hydrogen phosphate. The pH of these solutions was adjusted with 10% NH_4OH. The calcium nitrate solution was initially heated to 90 ° C, then the ammonium phosphate was added under continuous stirring (600 rpm). The addition rate of the second reagent was 0.1 mL / min, and was added with a peristaltic pump.

In the synthesis of HA-AL composite aqueous solution of 20 mM alendronate was added after the addition of ammonium hydrogen phosphate. The HA-AL 20 mM composite was deposited on Ti discs using MAPLE techique [8].

The scheme of the operations performed in the synthesis of the compound is presented in following figure:

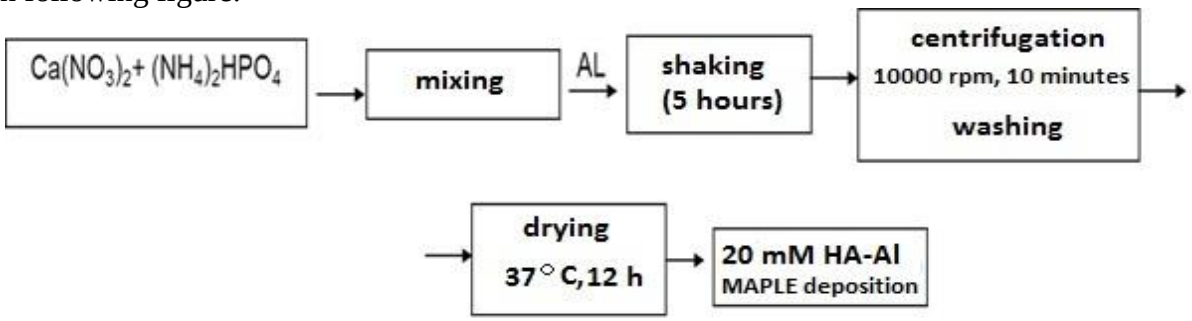

Figure 1. Scheme of hydroxyapatite-alendronate synthesis.

***In vitro* release profile of alendronate composite** - (HA-AL 20 mM) deposited on titanium by MAPLE tehnique was studied using ultrapure water release medium at a temperature of 37 ° C ± 0.5 °C (model oven). In a flat-bottomed flask equipped with a stopper to ensure tightness during the assays, 5 mL of ultrapure HPLC water (LiChrosolv, Merck) was added and then a 20 mM HA-AL coated titanium plaque was added, weighed in (m = 0.3328). The flask was sealed to avoid water vapor loss and placed in a preheated oven at a temperature of 37 ° C ± 0.5 °C. At regular intervals (1 h, 6 h, 10 h, 24 h, 48 h, 72 h, 96 h, 10 days, 21 days), 0.5 mL of the solution was taken for the quantitative determination of alendronate by HPLC. The volume removed was replaced by the release medium (ultrapure water).

HPLC analysis was performed by Thermo Finnigan Surveyor HPLC system equipped with a diode array UV-VIS detector and Thermo Finnigan Xcalibur data acquisition system. Separation was performed on a C18 reversed phase column Hypersil GOLD (Thermo Scientific) 250 mm x 4.6 mm I.D., 5 μm. The mobile phase used consists of an isocratic mixture of acetonitrile (65%) and 25 mM sodium citrate solution (35%) with a constant flow rate of 1 ml / min. The flow rate of the mobile phase was 1 mL / min, all experiments being performed at ambient temperature. Over 0.5 mL of the sample was added 0.5 mL of ultra-pure water and 5 drops of concentrated HCl to effect hydrolysis of the compound and the sample was left to stand for 10 minutes. 10 drops of ammonia were added to reprecipitate the hydroxyapatite, and then centrifuged at 6000 rpm for 6 minutes. 0.5 mL of 9-fluorenylmethyl derivative FMOC (2 mg / mL) and 1 mL of sodium citrate was added over the supernatant, then again was allowed to stand for 10 minutes at rest. It was brought to a total volume of 5 mL with ultrapure water, then 20 μL was injected into the HPLC system.

For the quantification of alendronate, a calibration curve was constructed in the concentration range of 0-50 mg / mL. Dilutions were done in ultra-pure water. The calibration curve obtained (the chromatographic peak area based on the analyte concentration) is linear in the concentration range of 3 - 50 mg / ml. The equation is: y = 59712516 x + 110871.1 with a correlation coefficient of 0.999311 (Fig. 1).

The retention time for alendronate was approximately 5.5 minutes with a peak absorption at 300 nm.

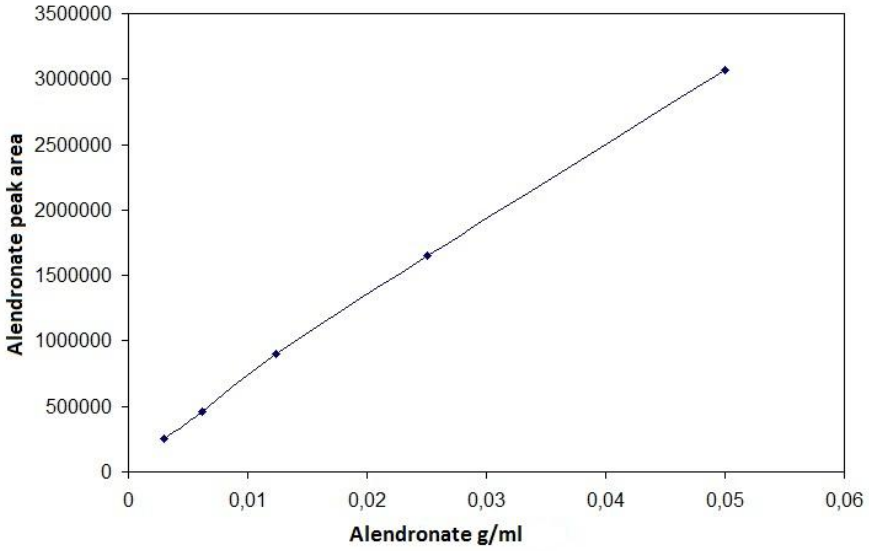

Figure 2. Calibration curve obtained by HPLC for alendronate determination.

3. RESULTS AND DISCUSSION

The blood flow that reaches the bone level is low and implicitly the concentration of drug administered either orally or injectable is reduced. Therefore, binding of alendronate to hydroxyapatite and deposition of the composite on the metallic surface of the implant may lead to an improvement in the alendronate sodium release profile, local release over a longer period of time preventing bone loss.

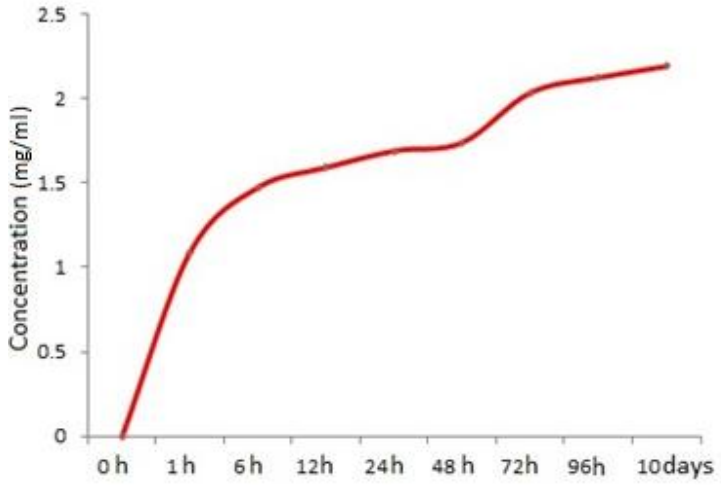

Figure 3. The amount of alendronate given (mg / mL) of 20 mM HA-AL deposited on Ti in the time unit.

Following the determinations, it was found that by synthesis of the hydroxyapatite (HA) - alendronate (AL) type composite and its deposition directly on the metallic component of the prosthesis by pulsed laser evaporation, a slow drug release occurred during the 10 days (Fig. 3).

The experiment was performed in triplicate, finding the same way of releasing alendronate. Fig. 4 shows the percentage of alendronate given as a function of the time unit.
Analyzing the graph (Fig. 4), it is noted that the largest amount of alendronate is given in the first hour after insertion of the metal disc into the release medium (67.27%), during the following 48 hours the yield is slow (79%) then there is a fairly rapid increase in alendronate release from 79% alendronate released 48 hours to 92% alendronate released at 96 hours. The final amount of alendronate is released at a slow rate over 6 days (from 92% to 100%).

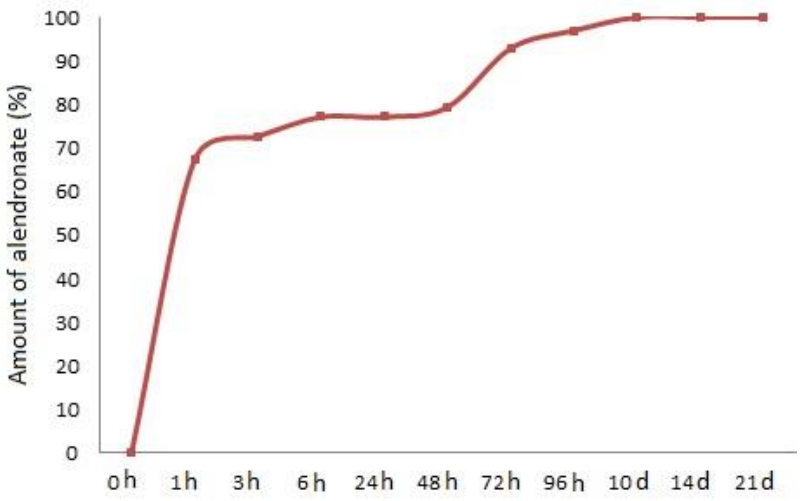

Figure 4. Percentage of alendronate given as a function of time.

Since HA-AL powder has been used in the present study primarily to obtain nanoscale nanoparticles by pulsed laser deposition, it has been found that these synthesized compounds by can be used without negative effects on cellular proliferation due to the presence of a low concentration of alendronate in the film. Alendronate released from the 20 mM HA-AL film falls within the range of values in which alendronate can be used in implantology without adversely affecting cell proliferation in the injured area.

4. CONCLUSIONS

From the experimental data, crystalline hydroxyapatite is a stable phase in contact with the release medium, and alendronate bound to HA through the synthesis process is slowly released from the metallic component (titanium) into the release medium over a period of 10 days, so in the case of implantation it contributes to bone resorption. Concomitantly, HA produces after implantation chemical species that support the implant's adhesion to the surrounding tissue forming a functional connective structure. HA amorphous and tricalcium phosphate are less stable and faster resorbed by the body. Thus synthesis of the HA-AL composite can be considered as a viable solution for the inclusion of bisphosphonate on the surface of metallic prosthetic components used in orthopedics.

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