Original Paper

Formulation and In Vitro Evaluation of Alendronate Sodium/PLGA Microspheres for Applications in Bone Related Disorders

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ABSTRACT: Purpose - Bisphosphonates are a group of drugs that can be used in the development of new therapies for bone disorders. Microencapsulation by solvent evaporation is a method that results in microspheres with controlled release of the drug. The purpose of the present study was to include sodium alendronate (AL) in poly (lactic-co-glycolic acid) (PLGA) microspheres with a good encapsulation rate. Furthermore it was intended to obtain a prolonged release of the drug over a period of time. Material and methods - Microspheres were prepared by water/oil/water solvent evaporation method. The microspheres were characterized by FTIR analysis using an Avatar Nicolet spectrophotometer. Both encapsulation ratio and in vitro drug release were assessed by chromatographic analysis. Results - The method chosen for sodium alendronate incorporation resulted in microspheres with a good entrapment efficiency. The in vitro drug release from PLGA/AL microspheres was maintained up to 21 days. This suggests that the microspheres had a sustained releasing tendency. Conclusion - Given the prolonged release, PLGA/AL microspheres may be used in a controlled release system with applications in bone disease treatment.

KEYWORDS: *alendronate sodium/PLGA microspheres, bone disorders, formulation, in vitro*

Introduction

Over the past few decades due to new developments in the biomaterial field and new surgical techniques it was reported an exponential increase in the research of medical delivery systems with appliance in restoring bone tissue integrity [1-4].

Bisphosphonates are highly potent inhibitors of bone resorption due to their affinity to hydroxyapatite. Furthermore they interact with the osteoclasts diminishing their activity. Also they enhance bone formation by raising the proliferation of osteoblasts and decrease the differentiation of osteoblasts into osteoclasts [1-3].

Due to their activity bisphosphonates can be used in the treatment of osteoporosis, Paget's disease, multiple myeloma, bone cancer, myeloproliferative disease and inflammation related to bone loss [3-5]. The presence of nonhydrolyzable P–C–P groups in the structure of bisphosphonates determines a low gastrointestinal absorption (0.7% for alendronate) (Fig 1) [6]. Only 20% of the absorbed drug is incorporated in the bones [6]. Furthermore the oral administration of bisphosphonates presents a series of side effects involving the gastrointestinal tract (esophageal irritation, ulcers and gastritis). Since 2003 it has also been linked with osteonecrosis of the jaws [3,4,7]. Due to its potential bisphosphonates could be used in combination with tissue-engineering techniques in restoring bone integrity [4]. Thus developing new biodegradable systems for drug delivery will minimize patient's discomfort and increase patient's compliance.

Using drug delivery systems (DDS) instead of active molecules reduces toxicity and also protects the drug from being inactivated. Microparticles are DDS that have a controlled drug release which results in higher drug efficiency. Also they improve bioavailability and reduce dosing frequency [7-9].

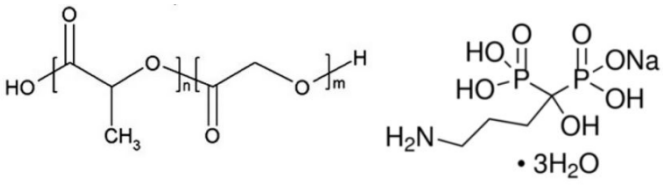

Fig 1. Chemical structures of poly (lactic-co-glycolic acid) and monosodium alendronate

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Microparticles can be obtained through a number of techniques, each exhibiting both advantages and disadvantages. Using solvent evaporation method will result in the synthesis of microparticles that release the drug slowly with a certain rate [9, 10]. Microencapsulation by solvent evaporation is adjusted to the hydrophobic or hydrophilic nature of the drug so that the encapsulation rate is maximized. Insoluble drugs are incorporated using oil-in-water (o/w) method whereas hydrophilic drugs such as sodium alendronate are incorporated by water-oil-water (w/o/w) double emulsion [9, 11].

Biodegradable polymers have high in vivo stability. Adjusting their degradation rate can be used to control the drug delivery rate. The most used polymers are polyanhydrides, poly lactic acid (PLA), poly glycolic acid (PGA) and their copolymer poly (lactic-co-glycolic acid) [12]. PLGA is a polymer approved by the FDA for its safe use in humans. It is both biocompatible and biodegradable [13]. PLGA degrades by hydrolysis into lactic acid and glycolic acid which are then eliminated through metabolic pathways. PLGA microspheres, due to their controllable degradability, have a large scale of applications. PLGA microspheres may be used to deliver both drugs and genes [12].

The aim of the study is to obtain PLGA microparticles with a high sodium alendronate concentration that will release the drug at a constant rate over a prolonged period of time that could be used as a new approach in the development of new therapies for metabolic bone disorders.

Material and methods

1.Materials

PLGA (65:35) was purchased from Sigma-Aldrich, polyvinyl alcohol (PVA) and sodium alendronate were purchased from Merck KGaA.

All the other chemicals (span 80, water) were of analytical grade.

2.Microencapsulation

PLGA microparticles were prepared by w/o/w solvent evaporation technique. 7 mg of sodium alendronate was dissolved into 170 mg of water forming the first aqueous phase W_1. The organic phase was formed by dissolving 160 mg PLGA in 3 ml methylene chloride. The primary emulsion (W_1/O) was formed by emulsifying the aqueous phase in the organic one using span 80 as an emulsifier at 30.000 rpm. The W_1/O emulsion was poured into 600 ml 0.1% PVA aqueous solution resulting into a W/O/W emulsion. This emulsion was stirred at 1000 rpm for 4 hours at room temperature to evaporate the solvent and obtain the PLGA/AL microspheres.

3.FTIR analysis

Fourier Transform Infrared Spectroscopy (FTIR) spectra for PLGA and PLGA/AL microspheres were recorded on an Avatar Nicolet spectrophotometer in KBr pellets, within the range 400-3000 cm^{-1}.

4.Encapsulation rate

The drug encapsulation efficiency was measured after extraction from the microspheres. 20 mg of microspheres were weighed and 5 ml methylene chloride was added to dissolve the polymer. Then 1 ml of sodium citrate solution was added to this phase and centrifuged at 6000 rpm for 10 minutes. The supernatant was analyzed by high-performance liquid chromatography (HPLC) with a Thermo Finnigan Surveyor HPLC System after derivatization with FMOC at a wavelength of 300 nm.

The drug encapsulation efficiency was calculated with:

Encapsulation efficiency (%) = (actual drug encapsulated/ theoretical drug encapsulated) x 100

5.Drug release study

To study drug release, nanoparticles were placed using MAPLE technique on a titanium disc 8 mm in diameter, double-sided polished. The disc was put in 5 ml aqueous solution and incubated at 37^{o}C. At predetermined time schedule, release medium was taken and the concentration of the released drug was measured by HPLC analysis.

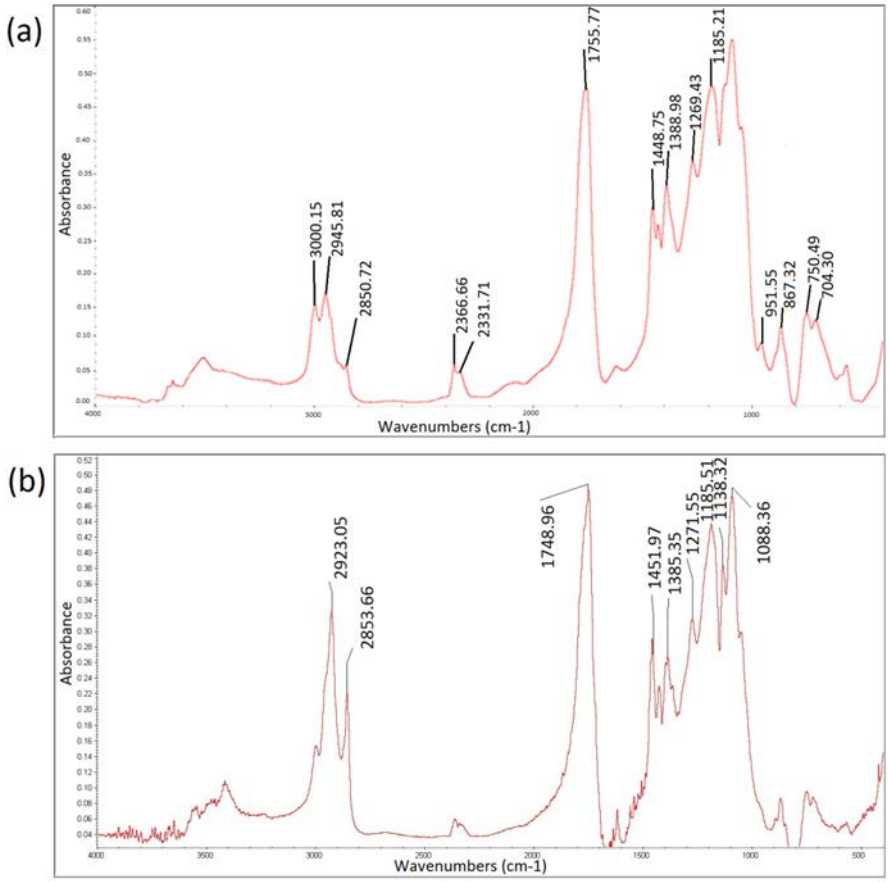

Fig.2. IR spectra of PLGA (a) and PLGA/AL microspheres (b)

Results

The IR spectra indicate an intense peak at 1750 cm^{-1} in both PLGA spectrum and nanoparticles spectrum, which is assigned to one of the characteristic peaks for PLGA, the carbonyl (C=O) stretching (Figure 2).

Peaks that are due to stretching of C-OH groups signal and the stretching of (CO)-OC groups can be observed in both PLGA and microsphere spectra, between 1300-1000 cm^{-1}. These groups are frequently present in PLGA structure (Fig. 1).

Figure 3 displays the in vitro release AL from PLGA microspheres at each time point. The microspheres are characterized by a biphasic release of the drug. The first part of the curve, from 0 to 48 h, corresponds to a burst release and it accounts for almost 70 percent of the released drug. Sodium alendronate was released from the microspheres over a 21 days period. The encapsulation efficiency found was of 13.24%.

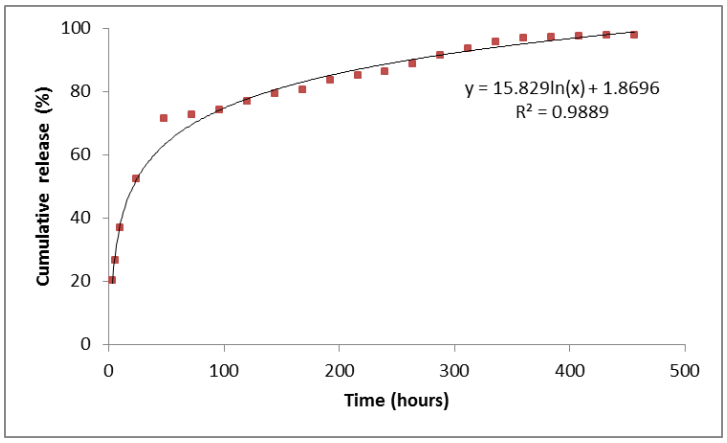

Fig.3. Cumulative release of sodium alendronate from microspheres

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Discussion

There is an intensive interest in the use of bisphosphonates in bone tissue engineering which requires a sustained release of the drug in the affected tissue. Coating the drug using biodegradable and biocompatible polymers shows great promise in obtaining a prolonged release of the incorporated drug [4].

Given that sodium alendronate is a hydrosoluble molecule, the double emulsion solvent evaporation technique (w/o/w) was chosen to encapsulate the drug. PLGA with a 65:35 polylactide and polyglycolide composition has been chosen for microspheres preparation due to its ability to form colloidal microparticle suspensions when using solvent evaporation method [1]. Microspheres with an encapsulation efficiency of 13.24% were obtained using this method. This percentage is slightly higher than the 7% observed from w/o/w methods [12, 14].

The release profile of the encapsulated drug can be modified by varying synthesis conditions, particle size, molecular weight, copolymer ratio and porosity. Biodegradation kinetics or interaction with encapsulated drug are chemical properties of the polymer that provide different possibilities for the design of controlled release systems [1]. As shown in Figure 2, it can be observed that the PLGA/AL microspheres obtained lead to a dissolution profile characterized by a biphasic drug release which is consistent with the studies performed so far [3]. We found the burst release phase (48 hours) to be longer then described in other studies where it is accounted for 24 hours [3]. In our experiment the burst release is due to the rapid release of the near surface drug. Once the microspheres are put in contact with the aqueous media, the sodium alendronate dissolves quickly. This phase is common for most controlled drug delivery systems. Then the dissolution rate decreases considerably following a zero order kinetic. This phase represents the release of the drug incorporated in the microparticles. The graphs show that the dissolution of the sodium alendronate occurs with a slow release at a near constant rate in the second part of the experiment. As shown in Figure 2, alendronate was continuously released over a three weeks period. Nafea et al reported that alendronate was released from PLGA microspheres over a period of 13 days [15]. In another study Samdancioglu et al. prepared sodium alendronate/PLGA microspheres by solvent evaporation using W/O/W emulsion with an entrapment efficiency of 7.7%. Furthermore sodium alendronate was 58% released in 5 days from the microspheres [16].

Conclusion

In this study sodium alendronate loaded PLGA microspheres were fabricated by double emulsion method. The microspheres showed good encapsulation efficiency (13.24%). The microspheres exhibited controlled release of the drug over a prolonged period although with an initial burst release. Therefore, as a favorable carrier of alendronate, PLGA microspheres are a promising multifunctional vehicle for bone repair.

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