Materials Science and Engineering C 63 (2016) 78–87

Contents lists available at ScienceDirect

Materials Science and Engineering C

journal homepage: www.elsevier.com/locate/msec

Risedronate/zinc-hydroxyapatite based nanomedicine for osteoporosis

Deepak Kumar Khajuria [a,b,*], Choudhary Disha [b], Ramakrishna Vasireddi [a], Rema Razdan [b], D. Roy Mahapatra [a]

[a] Laboratory for Integrative Multiscale Engineering Materials and Systems, Department of Aerospace Engineering, Indian Institute of Science, Bangalore 560012, India
[b] Department of Pharmacology, Al-Ameen College of Pharmacy, Bangalore 560027, India

ARTICLE INFO

Article history:
Received 26 July 2015
Received in revised form 15 February 2016
Accepted 20 February 2016
Available online 23 February 2016

Keywords:
Osteoporosis
Risedronate
Rat model
Zinc-hydroxyapatite
Nanomedicine

ABSTRACT

Targeting of superior osteogenic drugs to bone is an ideal approach for treatment of osteoporosis. Here, we investigated the potential of using risedronate/zinc-hydroxyapatite (ZnHA) nanoparticles based formulation in a rat model of experimental osteoporosis. Risedronate, a targeting moiety that has a strong affinity for bone, was loaded to ZnHA nanoparticles by adsorption method. Prepared risedronate/ZnHA drug formulation was characterized by field-emission scanning electron microscopy, X-ray diffraction analysis and fourier transform infrared spectroscopy. *In vivo* performance of the prepared risedronate/ZnHA nanoparticles was tested in an experimental model of postmenopausal osteoporosis. Therapy with risedronate/ZnHA drug formulation prevented increase in serum levels of bone-specific alkaline phosphatase and tartrate-resistant acid phosphatase 5b better than risedronate/HA or risedronate. With respect to improvement in the mechanical strength of the femoral midshaft and correction of increase in urine calcium and creatinine levels, the therapy with risedronate/ZnHA drug formulation was more effective than risedronate/HA or risedronate therapy. Moreover, risedronate/ZnHA drug therapy preserved the cortical and trabecular bone microarchitecture better than risedronate/HA or risedronate therapy. Furthermore, risedronate/ZnHA drug formulation showed higher values of calcium/phosphorous ratio and zinc content. The results strongly implicate that risedronate/ZnHA drug formulation has a therapeutic advantage over risedronate or risedronate/HA therapy for the treatment of osteoporosis.

© 2016 Elsevier B.V. All rights reserved.

1. Introduction

Osteoporosis is a bone incapacitating malady that causes about 9 million bone fractures every year [1]. The current treatments for osteoporosis have notable restrictions, including adequacy and long term safety issues [2]. Osteoporosis therapeutics have been monopolized by bisphosphonates, which avert further bone degradation in well-established osteoporosis. However, it was found that bisphosphonate therapy was unable to replenish the previously lost bone [2,3]. Moreover, bisphosphonates like risedronate (RIS) are not easily absorbed by the intestine and exhibit variable bioavailability. Therefore, high doses are required to be administered orally, which cause adverse event like esophagitis [2,4]. Hence, there is a distinct need to make improvements in the RIS therapy available for osteoporosis. Thus a delivery system that can improve the bone properties with reduced dose of RIS has motivated current research in the field of drug delivery.

Calcium phosphates compose the main inorganic component found in the bone matrix and teeth of humans and vertebrates. The mineral phase of the mammalian bone and teeth is commonly assimilated to synthetic hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$), however, biological apatites contrast from the stoichiometric ones in numerous features, including non-stoichiometry, crystals with dimensions in the nanoscale and low degree of structural order. Studies conducted in the past demonstrated that nano-sized hydroxyapatite (HA) facilitates the bioactivity of osteoblasts, to provide quicker bone regeneration [5–8]. Furthermore, the results of our previous study strongly suggest that novel drug formulation of HA nanoparticles loaded with risedronate sodium/zoledronic acid, is an ideal approach for the treatment of osteoporosis in a rodent model of established osteoporosis [9,10].

Zinc is a crucial element which is found in about every kind of cell in the human body, with bone containing the significant part of an entire body. Zinc invigorates bone growth and bone mineralization by initiation of osteogenesis of osteoblast cells aside from restraining osteoclastic resorption [11–13]. However, it should be noted that overdose or overexposure to zinc has cytotoxic effects. Such toxicity levels have been seen to occur at intake of more than 225 mg of zinc [14]. Numerous studies have exhibited that zinc-HA (ZnHA) significantly enhanced the bioactivity of HA [13,15]. Furthermore, studies involving zinc-substituted apatite containing low amount of zinc have been reported, and shown to enhance osteoblast response and reduction of osteoclast activity [16–18]. Nanocrystalline ZnHA may be loaded with active agents offering a perspective for providing innovative drug-delivery systems [19].

Recently, much consideration has been made to build up the new drug delivery systems with numerous advantages over the

* Corresponding author at: Laboratory for Integrative Multiscale Engineering Materials and Systems (iMEMS), Department of Aerospace Engineering, Indian Institute of Science (IISc), Bangalore 560012, India.
E-mail address: deepak_kumarkhajuria@yahoo.co.in (D.K. Khajuria).

http://dx.doi.org/10.1016/j.msec.2016.02.062
0928-4931/© 2016 Elsevier B.V. All rights reserved.

conventional dosage forms. Targeting is an attempt to deliver medication to a particular site and not to allow medication to diffuse to other locales where they may elicit serious side effects. It was found that under conditions likely to stimulate bisphosphonate binding onto bone, RIS has a strong binding affinity for HA [20]. In the periodic table, zinc shares the properties of group 2A elements with calcium, therefore zinc can supplant calcium in HA and subsequently in bone. Like HA, ZnHA is also expected to exert similar chemical influence and affinity for bisphosphonates. This prompted the investigation of bisphosphonates for targeting ZnHA to bone tissues [21]. Moreover, bisphosphonates after administration are quickly cleared from circulation and localize to the bone surface at the locales of active bone remodeling, specifically in the regions undergoing osteoclastic resorption [22]. Furthermore, recent studies have demonstrated that zinc containing β-tricalcium phosphate nanoparticles was highly effective in prevention of bone loss in ovariectomized mice/rat model and has potential uses in curing periodontitis [23,24]. On the other hand, a study comparing the efficacy of ZnHA nanoparticles used in combination with RIS in osteoporotic animals has not been previously reported. Therefore, in the present study, to achieve bone targeting of ZnHA nanoparticles, RIS was selected as a carrier due to its strong affinity to ZnHA.

There is an important need for naturalized therapeutics using novel bisphosphonate compounds that can act as both drug as well as a delivery vehicle for targeting bone and the surrounding soft tissues. The carrier itself will help in bone mass deposition and naturalized growth of bone tissue. This approach will allow selective treatment of bone related diseases while eliminating or minimizing the severe side effects previously seen with bisphosphonate therapies. The present study is in view of above idea which is quite novel. The proposed therapeutic intervention isn't just using a biocompatible ZnHA which itself acts as a drug, but additionally decreases the dose of the RIS.

2. Materials and methods

2.1. Materials

RIS was obtained as a gift sample from Fleming Laboratories Ltd., Hyderabad, India. Zinc nitrate hexahydrate, urea, ethylene diamine tetra acetic acid, ammonia solution, diammonium hydrogen phosphate, acetone, potassium dihydrogen phosphate, disodium hydrogen phosphate, sodium chloride, 4-nitrophenyl phosphate, sodium acetate, sodium tartrate, hydrochloric acid and xylene were purchased from S.D. Fine Chemicals, Mumbai, India. Ketamine was purchased from Neon Pharma, Mumbai, India and xylazine from Indian Immunologicals Ltd., Hyderabad. Ethicon chromic sutures-3/0, and Ethicon mersilk sutures-3/0 were purchased from Johnson & Johnson Ltd., Baddi, Himachal Pradesh, India. Povidone ointment was purchased from Cipla, India.

2.2. Fabrication of risedronate functionalized ZnHA nanoparticles

5 mol% ZnHA nanoparticles were prepared by substituting the calcium nitrate with zinc nitrate. Briefly, nanoparticles of ZnHA were synthesized from the initial molar ratio of (calcium + zinc)/phosphate at 1.67 with the 5 mol% initial zinc concentrations to the total (calcium + zinc). The obtained gel was then dried at 340 °C for 3 h under ambient static air. The gel was subsequently subjected to 900 °C calcinations from 300 to 900 °C at 10 °C/min, holding at 900 °C for 3 h, followed by cooling the products from 900 to 200 °C at 10 °C/min and then air-cooling to room temperature. The resultant solid of white powder was crushed using a mortar and pestle into a fine powder [24]. For field-emission scanning electron microscopy (FE-SEM) measurements, nanoparticles were mounted on metal stubs using double-sided adhesive tape, dried in a vacuum chamber, sputter-coated with a gold layer of 10 nm thick and viewed under high resolution FE-SEM (Ultra 55, Karl Zeiss Microscopy, Germany). FTIR spectroscopy analysis (Nicolet-Nexus 670 FTIR spectrometer, Nicolet Instrument Corporation, Madison, USA) was carried out to identify the functional groups. The spectrum was recorded in the 4000–400 cm^{-1} region with 2 cm^{-1} resolution. The crystallographic structural analysis was carried out by XRD method using a Bruker D8 Advance X-Ray powder diffractometer (Bruker, Germany) with monochromatic Cu Kα radiation over the 2Θ range of 0–80° at a scan rate of 0.02°/min.

Three unique proportions of RIS and ZnHA were taken, which are 1:1, 1:0.75, and 1:0.50, respectively (Table 1). Each RIS/ZnHA proportion was added into separate volumetric flask containing phosphate buffer with pH 7.4 and stirred with a magnetic stirrer for 7 h at 37 °C, the supernatant was analyzed every hour for RIS by UV spectrophotometer at 262 nm [9]. After stirring, the RIS/ZnHA samples were separated from drug loading medium by filtration, and dried. The process yields of ZnHA nanoparticles with diverse RIS-to-ZnHA proportions were determined using the formula: (Practical yield / Theoretical yield) × 100.

2.3. Determination of drug-loading capacity

About 10 mg of each dried RIS/ZnHA (RIS loaded nanoparticles of ZnHA) drug formulation was taken in 5 ml phosphate buffer saline (PBS) and ultrasonicated for 10 min. The supernatant was centrifuged for 10 min at 8000 rpm under 37 °C. The RIS content released in the formulation was determined by UV spectrophotometer at 262 nm. RIS/ZnHA proportion of 1:1 was chosen for pre-clinical study due to maximum % binding of RIS on ZnHA nanoparticles. Size of RIS/ZnHA nanoparticles (RIS-to-ZnHA 1:1) of drug was determined by FE-SEM, XRD and FTIR were used to identify the functional groups.

2.4. In-vitro release study

Around 100 mg of RIS/ZnHA drug formulation was transferred to a 100 ml beaker containing 50 ml of PBS pH of 7.4 and subjected to continuous stirring at 100 rpm 37 °C. Samples were withdrawn at predetermined time intervals, and the RIS content was analyzed by UV spectrophotometer at 262 nm. An equivalent volume of PBS supplanted the specimens that were withdrawn.

2.5. Animals

In-house research center bred healthy Wistar rats with 12 weeks age were used in this study. Animals were maintained under controlled temperature at 25 °C ± 2 °C with 12 h light/dark cycle with food and water and provided ad libitum. Ethical clearance was obtained from the Institutional Animal Ethical Committee (IAEC). The experiments were conducted as per the guidelines of the institutional ethics committee in a CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals).

2.6. Pre-clinical study design

To induce osteoporosis, ovariectomy was performed on female rats as indicated by the strategy described previously [25]. For the development of osteoporosis, ovariectomized (OVX) rats were left untreated for 12 weeks. Sham operations were performed by exteriorizing the ovaries. After 12 weeks of ovariectomy (age: 24 weeks), rats were divided

Table 1
Percentage yield and drug loading of ZnHA nanoparticles.

RIS-ZnHA ratio	RIS (mg)	ZnHA (mg)	Yield (%)	Drug loading (%)
1:1	300	300	89	62 ± 9
1:0.75	300	225	78	44 ± 7
1:0.50	300	150	62	37 ± 8

Data are shown as the mean ± SD, ($n = 3$).

Download English Version:

https://daneshyari.com/en/article/1427996

Download Persian Version:

https://daneshyari.com/article/1427996

Daneshyari.com