Available online at www.sciencedirect.com

ScienceDirect

journal homepage: http://www.elsevier.com/locate/aob

Ultrastructural and biochemical analysis of the effects of alendronate on salivary glands of young rats

Taís C. Oliveira, Vivian Bradaschia-Correa, Juliana R. Castro, Alyne Simões, Victor E. Arana-Chavez *

Department of Biomaterials and Oral Biology, School of Dentistry, University of São Paulo, Brazil

ARTICLE INFO

Article history:
Accepted 26 July 2014

Keywords:
Parotid gland
Submandibular gland
Alendronate
Amylase
Rat

ABSTRACT

Introduction: The bisphosphonates are drugs known by their antiresorptive potency and are widely prescribed for treating and preventing osteoporosis. In the past years the employment of this class of drugs had spread to other pathologies, and it is being prescribed to patients in a wide range of ages. Some adverse effects of bisphosphonate treatment have been highlighted recently, however, little is known about its potential side effects in salivary glands.

Methods: Newborn rats received daily doses of 2.5 mg/kg/day of sodium alendronate during 30 days. On the thirtieth day the animals were stimulated with pilocarpine and their parotid and submandibular glands were collected, fixed and embedded for histological and ultrastructural analysis. Some glands were collected for analysis of protein content and amylase activity.

Results: At light microscopy, the alendronate-treated animals presented an accumulation of secretion granules in their cytoplasm, which was confirmed by the ultrastructural examination. Biochemical analysis revealed an increase in total protein content and decreased amylase levels of both glands in the alendronate-treated animals in relation to the control.

Conclusion: Based on the current findings, alendronate is probably interfering in secretory pathways of parotid and submandibular glands.

1. Introduction

As other internal cavities in the body, the oral cavity has its surface lined by a mucous membrane to which salivary glands, a group of exocrine glands, flow their secretions for lubrication purposes. Due to its fluid characteristics, saliva is essential to preserve the integrity of teeth and oral soft tissues. In the oral cavity, however, saliva plays a more complex role than only lubrication. Normal salivary function is important for health maintenance. The decrease in salivary flow and changes in composition of saliva yield an imbalance that is manifested clinically by increased incidence of caries, susceptibility to oral candidiasis, xerostomia (dry mouth), difficulty speaking, chewing, swallowing, altered taste and halitosis.[1]

* *Corresponding author at*: Department of Biomaterials and Oral Biology, University of São Paulo School of Dentistry, Avenida Professor Lineu Prestes 2227, 05508-000 São Paulo, SP, Brazil. Tel.: +55 11 30917840; fax: +55 11 30917840.
E-mail address: vearana@usp.br (V.E. Arana-Chavez).

http://dx.doi.org/10.1016/j.archoralbio.2014.07.020
0003–9969/

The bisphosphonates are pyrophosphate-analogue drugs that are widely employed in prevention and treatment of osteoporosis and other bone diseases such as Paget's disease, multiple myeloma and bone metastasis of tumours. There are currently two major classes of bisphosphonates, the non-nitrogen containing (etidronate, clodronate) and the nitrogen-containing drugs (risedronate, alendronate and zoledronic acid), and the most prescribed is alendronate.[2] The effects of bisphosphonates in bone have been extensively studied, and their ability in inhibiting bone resorption by osteoclasts and thus interfering in bone remodelling. They are the most widely used class of antiresorptive drugs for the prevention and treatment of metabolic bone diseases, such as osteoporosis.[3] When administered systemically, the nitrogenated bisphosphonates are uptaken by clastic cells as they degrade the bisphosphonate-containing bone matrix[4] and induce several disturbs in their cellular metabolism and function. As they promote the inhibition of prenylation of the small GTPases Rho and Rab, their cytoskeletal dynamic and secretory functions are significantly altered.[5–7]

The effects of bisphosphonates on salivary gland secretory cells are unknown, despite the reports of the induction of a decrease of the resting salivary flow rate by osteoporotic patients under alendronate treatment[8] and stimulated whole saliva in patients under intravenous bisphosphonate therapy,[9] which can be detrimental to oral mucosa and teeth. Recently, it was demonstrated that patients treated with intravenous zoledronic acid present higher salivary levels of interleukin 1-α; the patients treated with zoledronic acid with signs of bisphosphonate-related osteonecrosis of the jaws present increase of several salivary markers of inflammation, such as interleukin-1α, interleukin-1 receptor antagonist and interleukin-1β[10] and also of markers of oxidative stress.[11]

Since some key molecules for osteoclast resorptive function like the small GTPases are also important for salivary secretory function,[12] it is expected that the structure and secretory function of salivary glands is affected by bisphosphonates. Lately, the bisphosphonates are prescribed to patients of different age ranges, including children with osteogenesis imperfecta and osteoporosis,[4] and their salivary glands may be a potential target of these drugs. We used an experimental model by which a high dose of sodium alendronate was daily administered to newborn rats from the day of birth until 30 days old[13] in order to evaluate its effects on PGs and SMGs.

The aim of the present study was to analyze the effects of a high dose of alendronate on the structure of the developing parotid and submandibular glands (PG and SMG, respectively), non-stimulated or stimulated with pilocarpine, a muscarinic sialogogue, at light and electron microscopy. In addition, biochemical analyzes were carried out for the determination of total protein concentration and amylase activity in stimulated glands under the conditions cited.

2. Materials and methods

2.1. Sodium alendronate treatment

Sixty newborn Wistar albino rats of both male and female sexes were used in this study. They were randomly divided into four groups: the control group non-stimulated with pilocarpine (CON group), the control pilocarpine-stimulated group (CONP group), the alendronate-treated non-stimulated with pilocarpine group (ALN group) and the alendronate-treated pilocarpine-stimulated group (ALNP group). Thirty rats, 20 from ALNP group and 10 from ALN group, were subjected to daily subcutaneous injections of 2.5 mg/kg/day sodium alendronate from the day of birth until 30 days old.[13] Additional thirty rats, 20 from CONP group and 10 from CON group, were daily injected with sterile saline solution during the same periods. All the alendronate-treated rats were not weaned during the entire study in order to have their nutrition provided maternally. Further time points were not included in the present study since the rat SMGs present gender dimorphism after 30 days.[14]

2.2. Sample obtaining and fixation

On the days cited, all rats were anesthetized with 2% chloridrate 2-(6,6-xilidine)-5,6-dihydro-4-H-1,3-tiazine diluted 1:1 in ketamine, 1 ml/kg body wt, and the group with stimulation received an intraperitoneal injection (7.5 mg/kg body wt) of pilocarpine to stimulate salivary secretion. Then, they were euthanized and had their parotid and submandibular glands dissected out and quickly processed as follows.

The salivary glands of 5 rats from each group were fixed in 0.1% glutaraldehyde + 4% formaldehyde, pH 7.4 for 6 h at room temperature and remained immersed in the fixative solution overnight at 4 °C.

2.3. Light microscopy

The specimens were dehydrated in graded concentrations of ethanol and embedded in JB-4 historesin. The 3 μm thick sections were stained with acid fuchsin and haematoxylin and coverslips were mounted with entellan. The sections were examined and photographed in an Olympus BX-60 light microscope by a blinded microscopist.

2.4. Transmission electron microscopy

Specimens of additional 5 rats from each group were fixed as described and then post-fixed in 0.1 M cacodylate-buffered 1% osmium tetroxide for 2 h at room temperature, dehydrated in graded concentrations of ethanol, and embedded in Spurr resin (Electron Microscopy Sciences, USA). Toluidine blue-stained 1-μm thick sections were examined in a light microscope and lobules of the glands were selected for ultrathin sectioning. 60-nm thick sections were obtained with a diamond knife on a Leica Ultracut R ultramicrotome (Leica, Buffalo, NY, USA), collected onto 200-mesh copper grids, stained with uranyl acetate and lead citrate and examined in a Jeol 1010 transmission electron microscope operated at 80 kV by a blinded microscopist. The images were digitally obtained with the GATAN imaging platform equipped with a SC1000 Orius CCD camera.

2.5. Biochemical analyses of protein content and amylase activity

Ten rats from the CONP group and 10 rats from ALNP group were used for the biochemical approaches (total protein