Available online at www.sciencedirect.com

ScienceDirect

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

Original Research Article

Investigation of microstructure of bone tissue in mandibles of newborn rats after maternal treatment with antiretroviral drugs

Jolanta Filipek [a], *Marcin Binkowski* [a,*], *Karina Maciejewska* [b], *Zofia Drzazga* [b], *Zygmunt Wróbel* [a]

[a] *X-ray Microtomography Lab, Department of Biomedical Computer Systems, Institute of Computer Science, Faculty of Computer and Materials Science, University of Silesia, Chorzów, Poland*

[b] *Chełkowski's Institute of Physics, Department of Medical Physics, University of Silesia, Katowice, Poland*

ARTICLE INFO

Article history:
Received 15 April 2014
Received in revised form
25 May 2014
Accepted 28 May 2014
Available online 16 June 2014

Keywords:
X-ray microcomputed tomography
Mandibular condyle
Antiretroviral drugs
Bone mineral density (BMD)
Histomorphometric parameters

ABSTRACT

High-resolution imaging has become a powerful tool for measurements in clinics, laboratory and animal studies, *etc*. In the present study, we aimed to investigate age related changes in bone development, and the effect of two antiretroviral agents (zidovudine and indinavir), which were administered during pregnancy, on the microstructure and bone mineral density (BMD) in newborn rats (7-, 14- and 28-day-old), with the use of X-ray microcomputed tomography (XMT). Fifty-four mandible bones were collected and divided into 3 groups: group 1 and 2: newborns after maternal treatment of zidovudine and indinavir respectively, group 3: control animals. The specimens were XMT scanned with the resolution of 7 μm and with a density phantom. Histomorphometric parameters and BMD were calculated to assess bone development depending on the administered drug. A statistical analysis was carried out to compare the differences among the control, zidovudine and indinavir groups. The analysis of the microstructure revealed disturbances in the development of the bone tissue in newborn rats. Indinavir seems to have a greater impact on bone microstructure than zidovudine.

1. Introduction

Advanced imaging methods have had an increasingly important role in preclinical investigations, especially in animal models, which can be used to elucidate the basic principles of the developmental origins of adult diseases. X-ray microcomputed tomography (XMT) allows to obtain high-resolution images and is an appropriate method for applications involving measurements of bone density and microstructure [1,2]. From among

* *Corresponding author at*: X-ray Microtomography Lab, Department of Biomedical Computer Systems, Institute of Computer Science, Faculty of Computer and Materials Science, University of Silesia, 75 Pułku Piechoty 1, Building H, Segment C, P.7, 41-500 Chorzów, Poland.
E-mail address: marcin.binkowski@us.edu.pl (M. Binkowski).

Abbreviations: XMT, X-ray microcomputed tomography; HIV, human immunodeficiency virus; ARV, antiretroviral treatment.

http://dx.doi.org/10.1016/j.bbe.2014.05.004

different hierarchical levels, from the ultrastructure of collagen to individual trabeculae [3], XMT allows to carry out measurements with the accuracy of 1 μm and assessment of the cancellous bone. Methods of quantification of bone architecture are based on reconstructed collection of 2D images [3]. XMT allows to determine 3D changes in the trabecular bone architecture, such as bone volume fraction (BV/TV), trabecular thickness (Tb.Th), trabecular number (Tb.N) and porosity (Po) [4]. Those histomorphometric parameters provide a quantitative analysis of the microscopic structure and organization of the bone tissue [5]. Bone volume over total volume (BV/TV) indicates the percentage of the mineralized bone which is located within the volume of interest (VOI). It can be used to evaluate relative changes in bone volume density following a given treatment [6]. Trabecular thickness (Tb.Th) is a good measure to compare the thickness of trabecular structures. Trabecular separation (Tb.Sp) can be expressed as the average distance among trabeculae. The average number of continuous trabeculae in a unit area or volume of sample is expressed with the trabecular number (Tb.N). Total porosity (Po) is the volume of all the open and closed pores as a percentage of the total volume [7].

Small animals such as rats are suitable for testing medicines, food products or cosmetics, for a number of reasons: frequent reproduction, genetic purity and similarities to human biology. Rat's mandibles are often analyzed in terms of the impact of various factors on bone density and structure [4,8–12].

Factors which may cause a reduced bone mineral density (BMD) and eventually bone fracture are commonly known [13]. Low BMD is widespread in HIV-positive population [14]. Women affected by this life-threatening disease who have decided to have offspring, need to undergo special treatment. Antiretroviral agents play a significant role in prevention of mother-to-child HIV transmission. Administration of antiretroviral drugs to the mother during pregnancy highly reduces the risk of perinatal transmission of HIV-1 infection [15]. Antiretroviral therapy is aimed at curing pregnant women, but it also brings a clear benefit to the fetus by reducing the risk of HIV transmission from about 40% [15]. However, adverse effects in mothers and children must be considered. For instance, zidovudine was related to momentary anemia [16] and potential carcinogenic effects in experimental animals [17]. It was reported that antiretroviral agents, such as tenofovir disoproxil fumarate (TDF), have a detrimental effect on bone growth and development in primates [18,19]. On the other hand, Mora et al. [20] concluded that ARV exposure during pregnancy does not affect negatively bone development in human infants, based on the measurement of the cortical bone with the use of quantitative ultrasonography (QUS).

Research related to bone development after maternal treatment with antiretroviral drugs had already been conducted. Drzazga et al. [21–23] determined bone mineral components and the contents of the organic matrix on the basis on fluorescence spectroscopy in newborn rats. Under UV and vis light excitation, biological tissues demonstrate the ability to reveal fluorescence. This technique is now commonly used as an imaging tool for oncological applications [24] and in diagnostics [25]. A lot of information about the condition of bone tissue can also be obtained from X-ray fluorescence spectroscopy. With the use of a miniature X-ray tube and a cryogenically cooled diode detector, it allows to perform non-invasive and non-destructive analysis. Concentrations of some elements present in the biological tissue (bone and teeth) during bone development and after maternal treatment with antiretroviral drugs were examined [21,26,27].

The aim of the present study was to analyze age related changes in bone microstructure in newborn rats (7-, 14- and 28-day-old rats). Rapidity and type of changes in bone density and microstructure of mandibular condyle in control group was compared to those of newborns after maternal treatment with antiretroviral agents: zidovudine and indinavir. It was hypothesized that antiretroviral drugs which were administered to the rats' mothers during pregnancy could cause disturbances in bone tissue in newborns. XMT as a high-resolution imaging technique allows estimation of parameters which describe bone microstructure. Together with the utilization of a calibration phantom, the measurement of bone mineral density in the mandibular condyle was determined. To our knowledge, there are no systematic studies on the influence of antiretroviral treatment on bone in newborns conducted with the use of XMT.

2. Materials and methods

2.1. *Specimen preparation*

The study was carried out on mandible bones from 7-, 14- and 28-day-old rats. The bones were divided into 3 groups ($n = 6$/group). The first and the second group consisted of

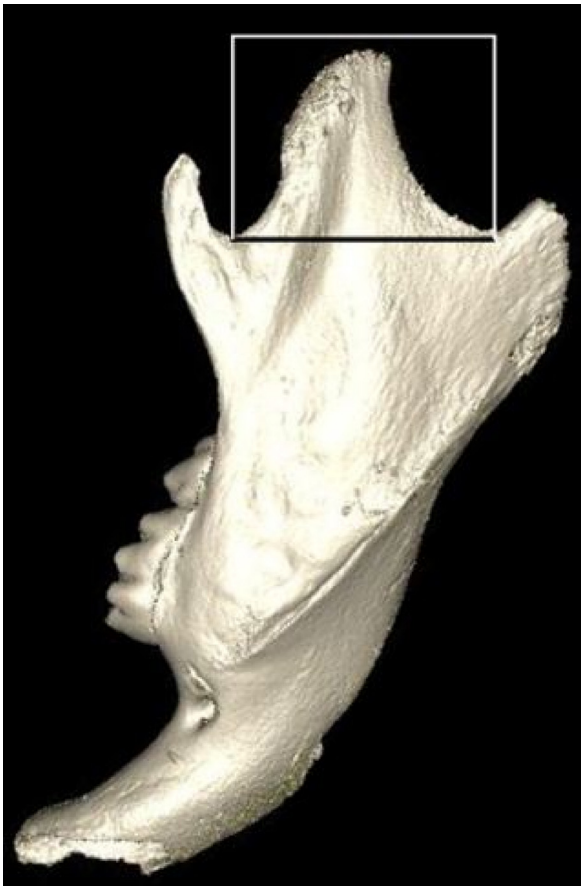

Fig. 1 – Three-dimensional visualization of the left mandible of a 28-day-old rat. In the box: region of interest during XMT scanning.