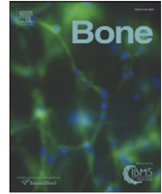
Bone 49 (2011) 1232–1241

Contents lists available at SciVerse ScienceDirect

Bone

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



Original Full Length Article

Lathyrism-induced alterations in collagen cross-links influence the mechanical properties of bone material without affecting the mineral

E.P. Paschalis [a,*], D.N. Tatakis [b,c], S. Robins [d], P. Fratzl [e], I. Manjubala [e], R. Zoehrer [a,1], S. Gamsjaeger [a], B. Buchinger [a], A. Roschger [a], R. Phipps [f], A.L. Boskey [g], E. Dall'Ara [h], P. Varga [h], P. Zysset [h], K. Klaushofer [a], P. Roschger [a]

[a] Ludwig Boltzmann Institute of Osteology at the Hanusch Hospital of WGKK and AUVA Trauma Centre Meidling, 1st Medical Department, Hanusch Hospital, Heinrich Collin Str. 30, A-1140 Vienna, Austria
[b] Division of Periodontology, The Ohio State University, Columbus, OH, USA
[c] Visiting Professor, King Saud University, Riyadh, Saudi Arabia
[d] Matrix Biochemistry, Rowett Institute of Nutrition and Health, University of Aberdeen, Aberdeen, Scotland, UK
[e] Max Planck Institute of Colloids and Interfaces, Department of Biomaterials, Research Campus Golm, Potsdam, Germany
[f] Dept. of Pharmacology, Husson University, ME, USA
[g] Hospital for Special Surgery, New York, NY, USA
[h] Institut für Leichtbau und Struktur-Biomechanik, TU Wien, Vienna, Austria

A R T I C L E I N F O

Article history:
Received 27 May 2011
Revised 24 August 2011
Accepted 26 August 2011
Available online 2 September 2011

Edited by: R. Baron

Keywords:
Collagen
Collagen cross-links
β-aminopropionitrile
Bone strength
Bone quality
Bone formation

A B S T R A C T

In the present study a rat animal model of lathyrism was employed to decipher whether anatomically confined alterations in collagen cross-links are sufficient to influence the mechanical properties of whole bone. Animal experiments were performed under an ethics committee approved protocol. Sixty-four female (47 day old) rats of equivalent weights were divided into four groups (16 per group): Controls were fed a semi-synthetic diet containing 0.6% calcium and 0.6% phosphorus for 2 or 4 weeks and β-APN treated animals were fed additionally with β-aminopropionitrile (0.1% dry weight). At the end of this period the rats in the four groups were sacrificed, and L2–L6 vertebra were collected. Collagen cross-links were determined by both biochemical and spectroscopic (Fourier transform infrared imaging (FTIRI)) analyses. Mineral content and distribution (BMDD) were determined by quantitative backscattered electron imaging (qBEI), and mineral maturity/crystallinity by FTIRI techniques. Micro-CT was used to describe the architectural properties. Mechanical performance of whole bone as well as of bone matrix material was tested by vertebral compression tests and by nano-indentation, respectively.

The data of the present study indicate that β-APN treatment changed whole vertebra properties compared to non-treated rats, including collagen cross-links pattern, trabecular bone volume to tissue ratio and trabecular thickness, which were all decreased ($p<0.05$). Further, compression tests revealed a significant negative impact of β-APN treatment on maximal force to failure and energy to failure, while stiffness was not influenced. Bone mineral density distribution (BMDD) was not altered either. At the material level, β-APN treated rats exhibited increased Pyd/Divalent cross-link ratios in areas confined to a newly formed bone. Moreover, nano-indentation experiments showed that the E-modulus and hardness were reduced only in newly formed bone areas under the influence of β-APN, despite a similar mineral content.

In conclusion the results emphasize the pivotal role of collagen cross-links in the determination of bone quality and mechanical integrity. However, in this rat animal model of lathyrism, the coupled alterations of tissue structural properties make it difficult to weigh the contribution of the anatomically confined material changes to the overall mechanical performance of whole bone. Interestingly, the collagen cross-link ratio in bone forming areas had the same profile as seen in actively bone forming trabecular surfaces in human iliac crest biopsies of osteoporotic patients.

© 2011 Elsevier Inc. All rights reserved.

* Corresponding author at: Ludwig Boltzmann Institute of Osteology, Hanusch Krankenhaus, Heinrich Collin Str. 30, A-1140 Vienna, Austria.
E-mail addresses: eleftherios.paschalis@osteologie.at (E.P. Paschalis), tatakis.1@osu.edu (D.N. Tatakis), s.robins@abdn.ac.uk (S. Robins), Peter.Fratzl@mpikg.mpg.de (P. Fratzl), i.manjubala@mpikg.mpg.de (I. Manjubala), Ruth.Zoehrer@flinders.edu.au (R. Zoehrer), sonja.gamsjaeger@osteologie.at (S. Gamsjaeger), birgit.buchinger@osteologie.at (B. Buchinger), andreas.roschger@osteologie.at (A. Roschger), PhippsR@husson.edu (R. Phipps), boskeya@hss.edu (A.L. Boskey), edallara@ilsb.tuwien.ac.at (E. Dall'Ara), vpeter@ilsb.tuwien.ac.at (P. Varga), philippe.zysset@ilsb.tuwien.ac.at (P. Zysset), klaus.klaushofer@osteologie.at (K. Klaushofer), paul.roschger@osteologie.at (P. Roschger).
[1] Current address: School of Chemical and Physical Sciences, Flinders University, GPO Box 2100, Adelaide, SA 5001, Australia.

8756-3282/$ – see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.bone.2011.08.027

Introduction

Historically osteoporosis has been defined as a disease in which there is "too little bone, but what there is, is normal" [1]. Although there is extensive data indicating this definition has to be modified [2], to date, working definitions of osteoporosis are based predominantly on bone mass. While evaluations of bone mass are of great clinical importance, they do not provide any information about the quality of the remaining bone mineral and matrix (in particular collagen) components [2].

The intermolecular cross-linking of bone collagen is intimately related to the way collagen molecules are arranged in fibrils. Six major collagen cross-links have been established as naturally occurring intermolecular cross-links. They are (i) dehydrodihydroxylysinonorleucine (deH-DHLNL) which exists primarily in its ketoamine form, hydroxylysine-5-keto-norleucine (HLKNL), (ii) dehydrohydroxylysinonorleucine (deH-HLNL) which is also present as the ketoamine, lysine-5-keto-norleucine (LKNL), (iii) pyridinoline (PYD), (iv) deoxypyridinoline (DPD; lysyl analog of PYD), (v) pyrroles (PYL and DPL), and (vi) histidinohydroxylysinonorleucine (HHL). The first two are reducible with borohydride (their reduced forms are referred to as DHLNL, and HLNL, respectively) and the rest are non-reducible compounds [3–6]. In mineralized tissue collagen the predominant cross-links are: HLKNL, LKNL, PYD, DPD, and pyrroles [7,8].

Data exist showing that the properties of collagen affect the mechanical strength of bone [9–11]. Recent clinical reports have correlated plasma homocysteine levels and bone fragility [12–15]. Homocysteine affects bone formation areas and in particular collagen cross-links [16]. The homocysteine-induced changes in collagen cross-links at trabecular bone forming and resorbing surfaces are similar to those seen in osteoporotic and fragility fracture patients [17,18]. Moreover, in a recent report employing spectroscopic analysis of iliac crest biopsies from 54 women (aged 30–83 yr; 32 with fractures, 22 without) who had significantly different spine but not hip Bone Mineral Density (BMD), it was found that cortical and cancellous bone collagen cross-link ratio strongly correlated positively with fracture incidence [19], further emphasizing the contribution of collagen cross-links in determining bone strength. In addition, in studies where there was a deviation between BMD values and bone strength, the spectroscopically determined pyridinoline (PYD)/divalent collagen cross-link ratio always correlated with bone strength [18–21]. One puzzling fact with these studies was the observation that the alterations in collagen cross-link ratio (PYD/divalent) were anatomically restricted to actively forming trabecular surfaces (based on either histologic stains or the presence of primary mineralized packets), while the rest of the bone seemed unaffected.

The purpose of the present study was to investigate whether anatomically confined alterations in collagen cross-links are sufficient to influence the mechanical performance of whole bone, employing the well-established β-aminopropionitrile (β-APN) treated rat model [22,23]. β-aminopropionitrile inhibits the lysyl oxidase-mediated formation of lysine aldehydes which are precursors of the major divalent and trivalent bone collagen cross-link moieties (HLKNL, LKNL, PYD, DPD). Vertebral bone was analyzed by μCT, micro finite element analysis (μFE), quantitative backscatter electron imaging (qBEI), compression mechanical testing, nanoindentation, and FTIRI analysis. Collagen cross-links were determined both chemically and spectroscopically.

Materials and methods

Animals

Under an Ohio State University IACUC-approved protocol, sixty-four female (47 day old) rats of equivalent weights were divided into four groups (16 per group): 2 control and 2 treatment groups. Controls were fed a semi-synthetic diet containing 0.6% calcium and 0.6% phosphorus as published elsewhere [24] for 2 or 4 weeks and β-APN treated animals were fed a diet inclusive of β-aminopropionitrile (0.1% dry weight) for 2 or 4 weeks . The 2 and 4 week-time points were used to allow formation of new bone with varying degrees of cross-linking in limited anatomical areas, without affecting the whole bone. Rats were sacrificed at the assigned time points and intact spines were harvested, dissected free from soft tissue and stored in 70% ethanol.

Chemical analysis of collagen cross-links

L3 vertebra from each animal were equilibrated with phosphate buffered saline, pH 7.8, pulverized and reduced with KBH_4 for 1 h. After this time, the pH was adjusted to 4 with acetic acid to destroy excess reagent, the tissue washed extensively with water and freeze dried. The reduced bone was hydrolyzed in 6 M HCl at 107 °C for 22 h and the acid was removed by evaporation. Following preliminary fractionation of cross-linked amino acids by partition chromatography, the intermediate compounds (DHLNL and HLNL) were assayed by ion-exchange chromatography with post-column derivatization and the mature bonds (PYD and DPD) were quantified using RP-HPLC using their natural fluorescence, as described previously [25]. Cross-link concentrations were expressed relative to collagen content determined by colorimetric measurement of hydroxyproline in the original hydrolysate. It should be noted here that both cortical and trabecular bone were included in the analysis.

μCT

The endplates of each L2 vertebra were carefully removed with a low speed diamond-coated saw (Isomet, Buehler, Germany) to provide samples with a height of approximately 3 mm. The vertebral body was then isolated from the posterior elements and one of the plane surfaces was glued on a carbon rod with a thin layer of cyanoacrylate glue. The other surface was polished to achieve parallel faces. The average final height was 2.5 mm. The vertebral body was scanned in 70% ethanol by μCT with a 12 μm voxel size (μCT40, Scanco, Switzerland). The reconstructions were segmented with an optimal threshold, separated into trabecular and cortical compartments and standard histomorphometric parameters were computed with the manufacturer's software (IPL, Scanco, Switzerland).

qBEI

Vertebrae (L5) were fixed in 70% ethanol, dehydrated through a graded series of ethanol and embedded undecalcified in polymethylmethacrylate (PMMA). About 5 millimeter thick blocks containing a sagittal vertebral bone section were cut using a low speed diamond saw (Buehler Isomed, Lake Pluff, USA). The section surface was ground with sand paper and subsequently polished by a diamond suspension (3 and 1 μm grain size, respectively) using a precision polishing device (MP5 Logitech, Ltd, Glasgow, Scotland). The sample surface was carbon coated prior to qBEI. A digital electron microscope (DSM 962, Zeiss, Oberkochen, Germany) equipped with a four quadrant semiconductor BE detector was used for backscattered electron imaging. The accelerating voltage of the electron beam was adjusted to 20 kV, the probe current to 110 pA, and the working distance to 15 mm. The digital backscattered (BE) images of trabecular bone areas were acquired by a single frame with a scan speed of 100 s/frame and a pixel resolution of 1 μm. Areas with high backscattered electron intensities – light gray levels – represent mineralized matrix with high Ca contents, whereas areas with low intensities – dark gray levels – indicate low mineral density. For the characterization and quantification of changes in the bone mineralization density distribution (BMDD) curve, four outcomes were used: CaMean (the weight mean calcium content of the bone area obtained from the integrated

Download English Version:

https://daneshyari.com/en/article/5891544

Download Persian Version:

https://daneshyari.com/article/5891544

Daneshyari.com