Biochemical and Biophysical Research Communications xxx (2016) 1–6

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Effect of glucocorticoid withdrawal on glucocorticoid inducing bone impairment

Gengyang Shen [a, 1], Hui Ren [a, 1], Ting Qiu [a], De Liang [b], Qiushi Wei [c], Jingjing Tang [b], Zhida Zhang [a], Zhensong Yao [b], Wenhua Zhao [a], Xiaobing Jiang [b, d, *]

[a] Guangzhou University of Chinese Medicine, Guangzhou, 510405, China
[b] Department of Spinal Surgery, The First Affiliated Hospital of Guangzhou University of Chinese Medicine, Guangzhou, 510405, China
[c] The First Affiliated Hospital of Guangzhou University of Chinese Medicine, Guangzhou, 510405, China
[d] Laboratory Affiliated to National Key Discipline of Orthopaedic and Traumatology of Chinese Medicine, Guangzhou University of Chinese Medicine, Guangzhou, 510405, China

ARTICLE INFO

Article history:
Received 4 July 2016
Accepted 7 July 2016
Available online xxx

Keywords:
GIOP
GC withdrawal
Bone impairment

ABSTRACT

Glucocorticoid (GC) withdrawal after a short-term use was common in clinical practice like immediate post-transplant period. However, previous studies without setting age-control group failed to determine whether the BMD recovery was sufficient and whether it is necessary to accept anti-osteoporosis therapy after GC withdrawal. The aim of this study was to investigate the effect of GC withdrawal on bone impairment in glucocorticoid-induced osteoporosis (GIOP) rats. Twenty-four female Sprague-Dawley rats (3 months' old) were randomly divided into two treatment groups: an untreated age-control group (Con, n = 12); another group receiving a dexamethasone injection (DEXA, n = 12). Animals in the Con group were euthanized at 3rd month (M3) and 6th month (M6), respectively. Six rats in the DEXA group were euthanized at 3rd month (M3), whereas GC intervention was withdrew in the remaining animals of DEXA group, which were euthanized at the end of 6th month (M6). Bone mass, bone microarchitecture, biomechanical properties of vertebrae, morphology, serum levels of PINP and β-CTX were evaluated. Compared with the Con(M3) group, the Con(M6) group showed significantly better bone quantity, morphology and quality. Compared with the Con(M3) group, the DEXA (M3) group showed significantly lower BMC, BMD, BS/TV, BV/TV, Tb.N, Tb.Th, vBMD, bone strength, compressive displacement, energy absorption capacity, PINP levels, β-CTX levels, and damaged trabecular morphology. And the same change trend was observed in the comparison between the Con(M6) group and DEXA (M6) group. Compared with the DEXA (M3) group, the DEXA (M6) group showed significantly higher BMC, BMD and AREA, but no significant difference in BS/TV, BV/TV, SMI, Tb.N, Tb.Th, Tb.Sp, vBMD, bone strength, bone stiffness, compressive displacement, energy absorption capacity, PINP levels, β-CTX levels, and improvement in trabecular morphology was observed. These results indicate that the reverse effect of GC withdrawal for 3 months on bone impairment in GIOP rats was insufficient, which implied that related anti-osteoporosis treatment might be still necessitated after GC withdrawal in clinical setting.

1. Introduction

Glucocorticoids (GCs) are frequently used to treat numerous diseases, such as rheumatoid arthritis, bronchial asthma and kidney transplant recipients etc [1–4], which also could induce deleterious effects on skeleton [5,6]. Simultaneously, GCs withdrawal after a short-term use was also common in clinical practice like immediate post-transplant period [7]. Recently, several researches showed that BMD could obtain a certain degree of recovery after

* Corresponding author. Department of Spinal Surgery, The First Affiliated Hospital of Guangzhou University of Chinese Medicine/Laboratory Affiliated to National Key Discipline of Orthopaedic and Traumatology of Chinese Medicine, Guangzhou University of Chinese Medicine, Guangzhou, 510405, China.
E-mail addresses: 15920198161@163.com (G. Shen), renhuispine@163.com (H. Ren), spinedrjxb@sina.com (X. Jiang).
[1] The co-first authors: Gengyang Shen and Hui Ren.

http://dx.doi.org/10.1016/j.bbrc.2016.07.036
0006-291X/

GCs withdrawal by self-contrast method [8,9]. But these studies without setting age-control group failed to determine whether the BMD recovery was sufficient and whether it is necessary to accept anti-osteoporosis therapy after GC withdrawal. Therefore, related study on the effect of GC withdrawal on bone impairment still needs to carry out.

Bone quantity is an important factor to evaluate skeletal health, but it could not replace the key role of bone quality in evaluating bone strength and bone microarchitecture. However, due to a series of restrictions, bone quality detected by micro-CT has not been widely popularized in clinical practice. Therefore, further basic study about bone quality in animal models is still required and this will provide an experimental basis for the clinical diagnosis, prevention and treatment for patients with GCs withdrawal. In addition, the changes of serum bone turnover markers and bone morphology after GCs withdrawal have not been reported in detail.

To further identify the effect of GC withdrawal on the bone impairment in the rat model, rats were treated with GC injection for three months, GC withdrawal for three months, and were compared with untreated age-control rats. Lumbar spine BMD, microarchitecture and biomechanical properties were measured to determine bone quality and quantity. HE staining was used to evaluate the trabecular bone morphology. Serum levels of amino-terminal propeptide of type I collagen (PINP) and β-C-telopeptide of type I collagen (β-CTX) were examined to evaluate bone turnover levels of GIOP rats after GC withdrawal.

2. Methods

2.1. Experimental animals grouping and study design

Female Sprague Dawley rats (n = 24) aged 3 month were purchased from Guangzhou University of Chinese Medicine and were maintained under standard laboratory conditions of controlled temperature (22–25 °C) and constant atmospheric pressure (25 kPa) in the animal room of the First Affiliated Hospital of Guangzhou University of Chinese Medicine (SYXK [Yue] 2013-0092) under a 12-hlight: dark cycle (lights on at 08:00). Food and water were available freely throughout the experiment.

After undergoing an acclimation period of one week, rats were randomly grouped into two as the untreated group (Con, n = 12), the dexamethasone intervention group (DEXA, n = 12), which were given a subcutaneous injection of dexamethasone-21-isonicotinate (batch no., H44022091; Guangzhou BaiyunshanTianxin Pharmaceutical Co., Ltd., Guangzhou, Guangdong, China) at a dose of 0.6 mg/kg body weight, twice per week for three months. In the Con and DEXA group, rats were euthanized randomly for experimental analysis at the end of the third month (M3, n = 6/group), and the remaining rats were euthanized in the sixth month (M6, n = 6/group).

Bone samples (lumbar vertebrae) devoid of soft tissues were isolated after euthanizing the rats. En bloc lumbar vertebrae 1–3 (L1-3) samples were stored at −20 °C for dual-energy X-ray absorptiometry. Lumbar vertebrae 2 (L2) samples obtained from L1-3 after BMD detection were fixed in 4% phosphate buffered paraformaldehyde for micro-CT and biomechanical analysis. Lumbar vertebrae 4 (L4) were transferred to 4% phosphate-buffered paraformaldehyde for histo-chemical analysis. Blood samples were collected and stored at −20 °C before assessment of bone turnover markers, including serum PINP and β-CTX.

2.2. Ethical approval

All the experimental procedures were approved by the ethic committee of the First Affiliated Hospital of Guangzhou University of Chinese Medicine (license no., 2,01,30,425). Humane care was provided according to the Guide for the Care and Use of Laboratory Animals, which was published by the US National Institutes of Health.

2.3. Bone mass measurement by dual-energy X-ray absorptiometry

The measured parameters include bone mineral content (BMC, g), bone mineral density (BMD, g/cm2), and bone area (AREA, cm2). The en bloc L1-3 samples were scanned by Dual-energy X-ray Absorptiometry (DXA) with a small-animal high-resolution collimator (Discovery A/SL/W/C; Hologic, Bedford, MA). After the scan, regions of interest were marked across the entire L1-3 region. Analysis was performed using the small animal mode of the software supplied with the collimator (v. 13.2:3; Hologic) and was calibrated at each start of the experiment.

2.4. Bone microarchitecture assessed by micro-computed tomography

L4 samples of the Con group and the DEXA group [n = 6/group (M3 and M6); n = 6/group (M3 and M6)] were scanned in a cone beam-type desktop micro-CT (μCT80; Scanco Medical, Brüttisellen, Zurich, Switzerland) and evaluated using the supplied software (μCT80 Evaluation Program v. 6.5–1; Scanco Medical). All target vertebrae were enclosed in a tightly fitting rigid plastic tube to prevent movement and positioned on a turntable that can be shifted automatically in the axial direction. In this study, the spatial resolution was set to 14 mm in all directions. After scanning, the cancellous bone of the vertebrae was chosen as the volume of interest, which was restricted to an internal region of the vertebrae where trabecular and cortical bones were extracted by drawing cylinder contours (diameter 2 mm) with the CT analyzer software.

The following parameters were measured: relative bone surface (BS/TV, mm-1), relative bone volume (BV/TV, %), structural model index (SMI), trabecular number (Tb.N, 1/mm), trabecular thickness (Tb.Th, mm), trabecular separation (Tb.Sp, mm), and volume bone mineral density (vBMD, mg HA/cm3). Three-dimensional images were obtained by multiplanar reformation.

2.5. Bone biomechanics evaluated by compression testing

To assess the mechanical quality of bones, a compression testing was carried out to determine the mechanical parameters with a material testing machine (ElectroPuls E1000 test system; Instron Corp., Norwood, MA) using L4 samples after micro-CT test. To obtain a central cylinder with planoparallel ends and height of approximately 5 mm, both end plates of the vertebrae body and its appendix were removed. The individual vertebrae were then tested along the longitudinal axis in the materials testing machine at a constant compression speed of 1 mm/min.

After the compression test, the load-displacement curve was plotted using the supplied software (Bluehill 3; Instron Corp.) to analyze the compressive strength (in N), compressive stiffness (in N/mm), compressive displacement (in millimeters), and energy absorption capacity (in Joules).

2.6. Trabecular bone morphology observed by HE staining

After removal of soft tissues from the surface, L4 of rats in each group was fixed in 4% paraformaldehyde, decalcified using 10% EDTA phosphate buffer, dehydrated and paraffin embedded using the conventional method, sliced to 5 μm sections, stained with hematoxylin-eosin and observed using Olympus-BH2 light microscope for trabecular bone morphology.

Please cite this article in press as: G. Shen, et al., Effect of glucocorticoid withdrawal on glucocorticoid inducing bone impairment, Biochemical and Biophysical Research Communications (2016), http://dx.doi.org/10.1016/j.bbrc.2016.07.036