Biochemical and Biophysical Research Communications xxx (2018) 1–6

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

A rat model of SHPT with bone abnormalities in CKD induced by adenine and a high phosphorus diet

Li-Hua Ni [a], Ri-Ning Tang [a, 1], Lin-Li Lv [a], Min Wu [a], Bin Wang [a], Feng-Mei Wang [a], Hai-Feng Ni [a], Kai-Yun Song [a], Li-Ting Wang [a], Meng-Zuo [a], Qiang Chen [b], Bi-Cheng Liu [a, *]

[a] Institute of Nephrology, Zhongda Hospital, Southeast University School of Medicine, Nanjing, Jiangsu, 210009, China
[b] Biomechanics Laboratory, School of Biological Science and Medical Engineering, Southeast University, Nanjing, Jiangsu 210096, China

ARTICLE INFO

Article history:
Received 4 March 2018
Accepted 6 March 2018
Available online xxx

Keywords:
Secondary hyperparathyroidism
Chronic kidney disease
Parathyroid hormone
Phosphorus
Adenine

ABSTRACT

The study of parathyroid hyperplasia with bone disease as a critical manifestation of chronic kidney disease-mineral and bone disorders (CKD-MBDs) is challenging due to the lack of a suitable research model. Here, we established a rat model with secondary hyperparathyroidism (SHPT) and bone disease induced by adenine and a high phosphorous diet and analyzed the skeletal characteristics. We performed blood analysis, emission computed tomography (ECT), dual energy X-ray absorptiometry (DEXA), micro-computed tomography (micro-CT), bone histomorphometry, and bone mechanical tests. The CKD rats with SHPT induced by adenine and a high phosphorus diet showed severe abnormalities in calcium and phosphorus metabolism and exhibited parathyroid hyperplasia. The bone mineral density (BMD) of femurs and lumbar vertebrae was significantly lower in the CKD rats than in the control (CTL) rats. The cortical and trabecular bone parameters of femurs showed significant bone loss. In addition, we found decreases in ultimate force, work to failure, stiffness, and elastic modulus in the CKD rats. In conclusion, our findings demonstrated that the CKD rats with SHPT induced by adenine and a high phosphorus diet may serve as a useful model for skeletal analysis in CKD with SHPT.

1. Introduction

Chronic kidney disease (CKD) is a worldwide health burden associated with high morbidity and mortality [1–4]. CKD is often complicated by the development of renal osteopathy due to disturbances in mineral metabolism, which are characterized by increased bone loss and bone fractures [5,6]. Secondary hyperparathyroidism (SHPT) is a common cause of renal osteopathy [7]. However, its detailed pathophysiology remains largely unknown. Consequently, developing a reliable rat model of osteoporosis with SHPT is of vital significance to study the basic aspects of this disease.

Exogenous adenine is immediately metabolized to 2,8-dihydroxyadenine, which precipitates and forms crystals in the microvilli and the apical region of the proximal tubular epithelia only 2 days after administration [8]. Increased crystal production induces degenerative changes in the cells of these tissues and causes renal dysfunction with increased levels of serum creatinine (Scr) and inorganic phosphate and decreased levels of serum calcium [9,10]. Many previous studies have reported that hyperphosphatemia in CKD leads to the increased progression of cardiovascular disease or vascular calcification, peripheral vascular disease, endothelial dysfunction, disorders of mineral and bone metabolism including bone fracture, and a higher mortality rate [11–13]. Recently, rat models of CKD with SHPT established by the administration of adenine and a high phosphorus diet were used to research vascular calcification and renal fibrosis [14–16]. However, a detailed analysis of the skeletal abnormalities in this model was insufficient.

In this study, we examined the skeletal system in an adenine-induced and high phosphorus-fed model of SHPT in CKD rats. Our data suggest that this is a reliable method for inducing severe SHPT with associated bone loss and chronic renal failure, which is more compatible with the clinical findings in kidney dialysis patients.

* Corresponding author. Institute of Nephrology, Zhong Da Hospital, Southeast University School of Medicine, No. 87 Dingjiaqiao Rd, Nanjing, Jiangsu, China.
E-mail address: liubc64@163.com (B.-C. Liu).
[1] Ri-Ning Tang is the co-first author.

https://doi.org/10.1016/j.bbrc.2018.03.038
0006-291X/

2. Methods

2.1. Animals

Study protocols were received and approved by the institutional animal care and use committee of Southeast University (Nanjing, China). Eight-week-old male Sprague Dawley rats (Animal Laboratory of Nantong University, China) were randomly assigned to two groups: (1) the control (CTL) group (n = 10) and (2) the CKD group (n = 10). CKD was induced by feeding 0.75% adenine for 4 weeks. After adenine withdrawal, all rats were maintained on a 1.5% phosphorus diet until the time of sacrifice at week 42. All diets were provided by Enuojia Feed Co., Ltd. (Nanjing, China).

2.2. Serum biochemistry

Serum creatine (Scr), blood urea nitrogen (BUN), and total calcium and phosphate concentrations were measured by a semi-automatic biochemical analyzer (ECA-2000A, Jilin, China) and UV-5100 spectrophotometer (Shanghai, China). Serum parathyroid hormone (PTH) determinations were performed via ELISA (MEIMIAN).

2.3. Emission computed tomography (ECT)

Parathyroid glands were scanned by tomography using a Siemens E-CAM SPECT instrument at the Southeast University-affiliated Zhongda Hospital. After using a minimum amount of anesthesia and avoiding artifacts due to animal movements, injection of $^{99m}TcO_4$-MIBI (0.37 MBq) was administered to rats. Images of the parathyroid glands were acquired at 15 min, 1 h, 2 h, 3 h, and 4 h.

2.4. Bone histology

For bone histopathology, rat tibias were isolated, and the soft tissue was carefully removed with a scalpel. Bone samples were fixed in 10% neutral buffered formalin and decalcified in 10% (w/v) ethylenediaminetetraacetic acid (EDTA). Bone samples were then embedded in paraffin, sectioned and subjected to hematoxylin and eosin (H&E) stain [17].

2.5. Bone mineral density (BMD) by dual energy X-ray absorptiometry (DEXA)

The BMD of femurs and lumbar vertebrae was measured by DEXA using a bone mineral analyzer (HOLOGIC, America) at the Southeast University-affiliated Zhongda Hospital.

2.6. Micro-computed tomography (micro-CT)

Using micro-CT (SkyScan 1176), the bone volume fraction, calculated as the ratio of the bone volume to tissue volume (BV/TV, %) and the bone architecture (number, spacing, and thickness) were determined from trabecular bone isolated from the metaphysis of the distal femur. The cortical bone geometry (area and thickness) was determined from the femoral midshaft. The metaphyseal scanning region of the intact femur consisted of 100 slices beginning 1 mm proximal to and extending away from the growth plate. The diaphyseal region was located halfway between the femoral head and distal condyles and consisted of 100 slices around the center. A scanning resolution of 18 μm was chosen for all animals, a minimum amount of anesthesia was used and artifacts due to animal movements were avoided. Data were analyzed using CT-Analyzer (SkyScan software). Three dimensional (3D) images and animations were created using CtVox (SkyScan software). Micro-CT parameters are reported according to international guidelines [18].

2.7. Bone mechanics

Structural mechanical properties and apparent material properties were assessed by uniaxial compression testing of the L5 vertebra (Instron 5943, America) as previously described. Prior to mechanical testing, the vertebral arch and endplates were removed. Samples were loaded at a rate of 0.5 mm/min, producing a force-displacement curve for each sample, and structural mechanical properties were obtained directly from these curves as previously described [19].

2.8. Statistical analysis

Data are expressed as the mean ± standard deviation (SD). Statistical analyses were conducted via one-way ANOVA by SPSS 21.0 statistical software. A value of $P < 0.05$ was considered to be statistically significant.

3. Results

3.1. Renal function and biochemical parameters

The biochemical data of the rats at 42 weeks of age are shown in Fig. 1. Compared with the CTL rats, rats in the CKD group developed severe renal failure, as evidenced by a significant increase in their Scr levels (462.82 ± 91.29 μmol/l vs 42.75 ± 9.48 μmol/l, $P<0.05$, Fig. 1d). Serum calcium levels were lower (2.28 ± 0.25 mmol/l vs 2.52 ± 0.26 mmol/l, $P<0.05$), whereas the levels of serum phosphorus (3.69 ± 0.16 mmol/l vs 2.72 ± 0.16 mmol/l, $P<0.05$) and PTH (542.94 ± 98.51 pg/ml vs 75.90 ± 12.10 pg/ml, $P<0.05$) were higher in the uremic rats than in the CTL rats (Fig. 1a–c, $P<0.05$).

3.2. Parathyroid hyperplasia

Next, we assessed parathyroid function by ECT (Fig. 2). Normal parathyroid gland functions were observed in the CTL group. However, the radiation aggregation at 3 h indicated parathyroid adenoma with hyperparathyroidism in the CKD group. Elevated serum PTH levels and ECT evidence confirmed the success of the SHPT model in CKD rats.

3.3. Bone histology and histomorphometry

To further describe the bone disorders, we performed H&E staining of rat tibias at the time point of 42 weeks (Fig. 3a). Well-formed, normal, connected and mature bony trabeculae with a benign bone thickness were observed in the CTL group. However, widely separated, thin-walled trabecular bone containing bone marrow elements were observed in the CKD group, suggesting osteoporosis.

BMD measurements by DEXA are shown in Fig. 3b. The BMD of femurs and lumbar vertebrae were significantly reduced in the CKD group compared with that in the CTL group (femur: 0.216 ± 0.016 g/cm^2 vs 0.255 ± 0.019 g/cm^2, $P<0.05$; vertebra: 0.294 ± 0.033 g/cm^2 vs 0.362 ± 0.040 g/cm^2, $P<0.05$).

The trabecular and cortical bone parameters measured by micro-CT are shown in Fig. 3c–j. The CKD animals had lower trabecular and cortical bone parameters than the CTL animals. In the distal femur, the BV/TV (29.387 ± 5.130% vs 44.248 ± 9.992%, $P<0.05$), the trabecular thickness (Tb.Th, 0.102 ± 0.010 mm vs 0.119 ± 0.015 mm, $P<0.05$), and the trabecular number (Tb.N, 2.908 ± 0.402 1/mm vs 3.577 ± 0.730 1/mm, $P<0.05$) were

Please cite this article in press as: L.-H. Ni, et al., A rat model of SHPT with bone abnormalities in CKD induced by adenine and a high phosphorus diet, Biochemical and Biophysical Research Communications (2018), https://doi.org/10.1016/j.bbrc.2018.03.038