Bone 58 (2014) 168–176

Contents lists available at ScienceDirect

Bone

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

Original Full Length Article

Effects of unfractionated heparin on renal osteodystrophy and vascular calcification in chronic kidney disease rats

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ARTICLE INFO

Article history:
Received 21 February 2013
Revised 6 October 2013
Accepted 14 October 2013
Available online 18 October 2013

Edited by: Rene Rizzoli

Keywords:
Chronic kidney disease
Renal osteodystrophy
Vascular calcification
Unfractionated heparin
Secondary hyperparathyroidism

ABSTRACT

Unfractionated heparin (UFH) is the most widely used anticoagulant in hemodialysis for chronic kidney disease (CKD) patients. Many studies have verified that UFH can induce bone loss in subjects with normal bone, but few have focused on its effect on renal osteodystrophy. We therefore investigated this issue in adenine-induced CKD rats. As CKD also impairs mineral metabolism systemically, we also studied the impacts of UFH on serum markers of CKD–mineral and bone disorder (CKD–MBD) and vascular calcification. We administered low and high doses of UFH (1 U/g and 2 U/g body weight, respectively) to CKD rats and compared them with CKD controls. At sacrifice, the serum markers of CKD–MBD did not significantly differ among the two UFH CKD groups and the CKD control group. The mean bone mineral densities (BMDs) of the total femur and a region of interest (ROI) constituted of trabecular and cortical bone were lower in the high-dose UFH (H-UFH) CKD group than in the CKD control group ($P<0.05$ and $P<0.01$, respectively). The BMD of the femoral ROI constituted of cortical bone did not differ between the H-UFH CKD group and the CKD control group. Histomorphometrical changes in the CKD rats indicated secondary hyperparathyroidism, and the femoral trabecular bone volume, but not cortical bone volume, significantly decreased with increasing UFH dose. The same decreasing trend was found in osteoblast parameters, and an increasing trend was found in osteoclast parameters; however, most differences were not significant. Moreover, no distinct statistical differences were found in the comparison of vascular calcium or phosphorus content among the CKD control group and the two UFH CKD groups. Therefore, we concluded that UFH could induce bone loss in CKD rats with secondary hyperparathyroidism, mainly by reducing the trabecular volume and had little effect on cortical bone volume. The underlying mechanism might involve inhibition of osteoblast activity and promotion of osteoclast activity by UFH. We did not find any effect of UFH on vascular calcification in CKD rats with secondary hyperparathyroidism.

Introduction

Hemodialysis has become the most widely used therapy for end-stage chronic kidney disease (CKD) patients in most areas of the world in recent decades [1]. Although it was first applied in humans in the 1920s, extensive application was hampered by the lack of a suitable anticoagulant to keep the blood flowing smoothly in the circuit. It was not until the 1940s, with the introduction of unfractionated heparin (UFH) in dialysis, that the practical use of hemodialysis became possible in clinical practice [2]. Since then, UFH has remained the most widely used anticoagulant in hemodialysis because of its flexibility, short half-life and low cost [3,4].

Abbreviations: BFR/BS, bone formation rate (bone surface referent); BFR/BV, bone formation rate (bone area referent); BMD, bone mineral density; BUN, blood urea nitrogen; CKD, chronic kidney disease; CKD–MBD, chronic kidney disease–mineral and bone disorder; Ct.B.Ar, cortical bone area (porosity area reduced); DXA, dual-energy X-ray absorptiometry; ELISA, enzyme-linked immunosorbent assay; FGF-23, fibroblast growth factor-23; GPWi, growth plate width; H-UFH, high-dose unfractionated heparin; ICP-OES, inductively coupled plasma optical emission spectrometer; KDIGO, Kidney Disease: Improving Global Outcomes; LGR, longitudinal growth rate; L-UFH, low-dose unfractionated heparin; MAR, mineral apposition rate; MLT, mineralization lag time; N.Ob/BS, osteoblast number per millimeter bone perimeter; N.Oc/BS, osteoclast number per millimeter bone perimeter; Ob.S/BS, osteoblast surface; Oc.S/BS, osteoclast surface; OS/BS, osteoid surface; OV/BV, osteoid volume; Po.Ar%, porosity area percent; Po.Ar, porosity area; primary BV/TV, primary spongiosa bone volume; PTH, parathyroid hormone; QCT, quantitative computed tomography; ROI, region of interest; secondary BV/TV, secondary spongiosa bone volume; SPA, single photon absorptiometry; Tb.N, trabecular number; Tb.Sp, trabecular separation; Tb.Th, trabecular thickness; UFH, unfractionated heparin.

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http://dx.doi.org/10.1016/j.bone.2013.10.010

UFH has several adverse effects, including heparin-induced osteoporosis. In 1965, Griffith et al. first reported an association between UFH usage and osteoporosis [5]. Subsequently, many clinical studies and animal experiments have reached the same result, and have shown that the extent of osteoporosis is related to the dose of UFH and the duration of administration [6–11]. However, while the bones of the subjects in these studies (mainly normal rats, pregnant women or patients with venous thromboembolism) were normal prior to heparin administration, the skeletal condition of CKD patients has been impaired before heparin administration in hemodialysis. On the one hand, most CKD patients have suffered renal osteodystrophy to different degree before UFH use in hemodialysis, because many influencing factors on renal osteodystrophy have existed in the early stage of CKD, such as hyperphosphatemia, hyperparathyroidism and calcitriol deficiency. On the other hand, even though they begin to use UFH continually in hemodialysis sessions at end-stage CKD, their skeleton would still be influenced continuously by the same factors besides heparin. Thus, the effects of heparin on bone in CKD patients are more complicated. We therefore aimed to determine whether UFH influenced renal osteodystrophy in CKD patients and if so, how it did.

Some epidemiological studies have found that patients with osteoporosis were at an increased risk of coronary artery and aorta calcification, not only in postmenopausal women and general elderly people [12,13], but also in CKD patients [14,15]. A study of a knock-out mouse model also indicated this relationship [16]. In this model, osteoporosis and arterial calcification developed simultaneously after the osteoprotegerin gene was knocked out in mice. These findings from basic research and clinical data brought the scientific community to change the nosology of CKD-related bone disorders. In 2005, the Kidney Disease: Improving Global Outcomes (KDIGO) foundation proposed the new nomenclature chronic kidney disease–mineral and bone disorder (CKD–MBD) to describe the syndrome of CKD incorporating abnormalities in serum markers of mineral metabolism, vascular calcification and renal osteodystrophy [17]. Based on the above information, we wondered in addition to influencing bone metabolism if UFH also had effects on vascular calcification and serum mineral markers in CKD patients.

In this study, we investigated the effects of UFH on serum markers of CKD–MBD, renal osteodystrophy and vascular calcification in an adenine-induced CKD rat model [18,19].

Materials and methods

Materials

UFH was purchased from No. 1 Biochemical & Pharmaceutical Co., Ltd. (Shanghai, China). Two different isocaloric synthetic diets were customized from Keao Xieli Feed Co., Ltd. (Beijing, China), modified from the AIN-93 standard purified diet [20], with each diet containing 9% protein (casein), 1.11% calcium, 1.03% phosphorus and 1000 IU/kg vitamin D3. 0.75% adenine was added to the second diet but not to the first one. Forty 13-week-old male Sprague–Dawley rats were purchased from Vital River Laboratories (Beijing, China).

Animal procedures

The CKD model was established by feeding an adenine and high phosphorus diet to rats [18,19]. All animals received humane care according to the Institutional Authority for Laboratory Animal Care of Peking University Health Center. After 2 weeks of adaptation, the rats were randomly assigned into four groups with 10 rats in each group. Two UFH doses (1 U/g body weight and 2 U/g body weight, respectively) were given to the CKD rats and these rats were compared with the CKD controls. The doses were determined on the basis of the studies of Muir et al. and other authors [6,7,9]. Fig. 1 shows the experimental setup. Body weights were determined at the initiation of the experiment and

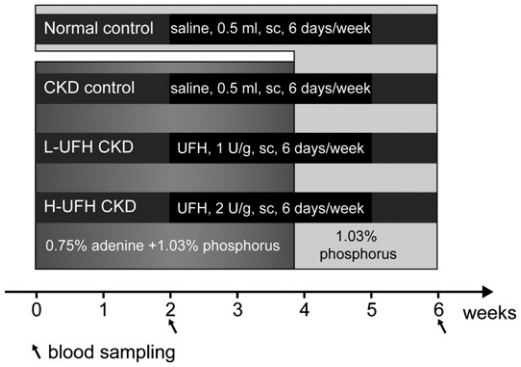

Fig. 1. Experimental setup. Forty rats were randomly divided into four groups of 10: (1) normal control, normal control group, saline, sc, the 3rd, 4th and 5th weeks; (2) CKD control, CKD control group, saline, sc, the 3rd, 4th and 5th weeks; (3) L-UFH CKD, low-dose UFH CKD group, UFH, 1 U/g body weight, sc, the 3rd, 4th and 5th weeks; (4) H-UFH CKD, high-dose UFH CKD group, UFH, 2 U/g body weight, sc, the 3rd, 4th and 5th weeks. The normal control group was fed a diet without adenine for all 6 weeks. The other three groups were fed an adenine-containing diet for 4 weeks followed by a diet without adenine for 2 weeks. CKD, chronic kidney disease; sc, subcutaneous; L-UFH, low-dose unfractionated heparin (UFH); H-UFH, high-dose UFH.

monitored twice every week to adjust the heparin doses. At the end of the second week (just before heparin administration), fasting blood was drawn from the retro-orbital plexus for biochemical tests under anesthesia. At sacrifice, animals were euthanized by exsanguination through the retro-orbital sinus under anesthesia and fasting blood was collected for biochemical tests. Through a combined midline laparotomy, the aortic vascular tree was exposed and the large vessel was removed entirely. The aortic segment from the beginning of the descending aorta to the abdominal aorta terminal was divided into two sections at a site just proximal to the celiac trunk. The proximal half was prepared for histology and the distal half was used to determine tissue calcium and phosphorus contents. Afterwards, the right femurs and fifth lumbar vertebrae were collected and stored at −20 °C prior to bone density tests and the left femurs were collected for bone histomorphometry. For dynamic parameters, rats were injected subcutaneously with calcein (10 mg/kg) at 13, 12, 3, and 2 days before necropsy.

Serum biochemical assays

Serum creatinine, blood urea nitrogen (BUN), calcium and phosphorus were measured at the clinical laboratory of Peking University First Hospital using a HITACHI 7170S analyzer (Tokyo, Japan). Serum parathyroid hormone (PTH) was tested using rat intact PTH enzyme-linked immunosorbent assay (ELISA) kits (Immutopics, San Clemente, CA, USA) and serum fibroblast growth factor-23 (FGF-23) was tested using rat FGF-23 ELISA kits (Merck Millipore, St. Charles, MO, USA).

Assessment of aortic calcification

Our methods for evaluating aortic calcification were modified from those of Neven et al. [21]. The proximal part of the aorta was cut into rings of approximately 2 mm wide before fixation in neutral buffered formalin. After full fixation, all rings from the same aorta were embedded upright in 3–4 paraffin blocks separately. Eight-micrometer sections were stained for calcification with Von Kossa's method and counterstained with eosin. Then vascular calcification was quantified histologically using Image-Pro Plus 5.1 image analysis software (Media Cybernetics, Silver Spring, MD, USA). The absolute areas of total tissue and calcified tissue were summed for each animal and the ratio was expressed as the percentage of aortic calcified area. The distal aorta samples were dried at 65 °C for 72 h and then weighed with a precision balance. Subsequently, tissue calcium and phosphorus contents were measured by inductively coupled plasma optical emission spectrometer