Bone 110 (2018) 244–253

Contents lists available at ScienceDirect

Bone

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

Full Length Article

PTH (1–34) and growth hormone in prevention of disuse osteopenia and sarcopenia in rats

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ARTICLE INFO

Article history:
Received 21 December 2017
Revised 31 January 2018
Accepted 19 February 2018
Available online 20 February 2018

Keywords:
Immobilization
PTH
GH
MicroCT
Biomechanics
Histomorphometry

ABSTRACT

Osteopenia and sarcopenia develops rapidly during disuse. The study investigated whether intermittent parathyroid hormone (1–34) (PTH) and growth hormone (GH) administered alone or in combination could prevent or mitigate disuse osteopenia and sarcopenia in rats.

Disuse was achieved by injecting 4 IU botulinum toxin A (BTX) into the right hindlimb musculature of 12–14-week-old female Wistar rats. Seventy-two rats were divided into six groups: 1. Baseline; 2. Ctrl; 3. BTX; 4. BTX + GH; 5. BTX + PTH; 6. BTX + PTH + GH. PTH (1–34) (60 μg/kg/day) and GH (5 mg/kg/day). The animals were sacrificed after 6 weeks of treatment. Sarcopenia was established by histomorphometry, while the skeletal properties were determined using DXA, μCT, mechanical testing, and dynamic bone histomorphometry. Disuse resulted in lower muscle mass (−63%, $p < 0.05$), trabecular BV/TV (−28%, $p < 0.05$), Tb.Th (−11%, $p < 0.05$), lower diaphyseal cortical thickness (−10%, $p < 0.001$), and lower bone strength at the distal femoral metaphysis (−27%, $p < 0.001$) compared to Ctrl animals. PTH fully counteracted the immobilization-induced lower BV/TV, Tb.Th, and distal femoral metaphyseal strength. GH increased muscle mass (+17%, $p < 0.05$) compared to BTX, but did not prevent the immobilization-induced loss of bone strength, BV/TV, and cortical trabecular thickness. Combination of PTH and GH increased distal femoral metaphyseal bone strength (+45%, $p < 0.001$), BV/TV (+50%, $p < 0.05$), Tb.Th (+40%, $p < 0.05$), and whole femoral aBMD (+15%, $p < 0.001$) compared to BTX and muscle mass (+21%, $p < 0.05$) compared to BTX + PTH.

In conclusion, PTH and GH in combination is more efficient at preventing the disuse-related deterioration of bone strength, density, and micro-architecture than either PTH or GH given as monotherapy. Furthermore, GH, either alone or in combination with PTH, attenuated disuse-induced loss of muscle mass. The combination of PTH and GH resulted in a more effective treatment than PTH and GH as monotherapy.

1. Introduction

Disuse and skeletal unloading such as bed rest and spinal cord injury results in a rapid and substantial bone loss [1–3] caused by an upregulation of genes associated with bone resorption and muscle atrophy and downregulation of genes associated with bone formation [4,5]. One of the most striking clinical effects of immobilization is an increased risk of bone fractures due to the mechanical unloading [6–8]. Moreover, disuse leads to a rapid and pronounced loss of muscle mass [9–11]. Different animal models have been proposed to investigate the effect of disuse including neurotoxic agents like botulinum toxin (BTX) [12,13], neurectomy [14,15], tail-suspension [16], and elastic bandaging [17]. In the present study, disuse was achieved by injecting BTX into the right hindlimb musculature [13]. BTX inhibits release of acetylcholine from the motor neurons into the synaptic cleft of the neuromuscular junction, which paralyze the musculature leading to a rapid loss of muscle and bone [9].

In order to counteract the rapid bone loss accompanying disuse, antiresorptive agents such as bisphosphonates [18,19] or bone anabolic agents such as intermittent parathyroid hormone (1–34) (PTH) [15,20–22] can be applied. However, disuse not only results in osteopenia, but also in a massive loss of muscle mass [9,23–25]. Consequently, in addition to prevent disuse induced osteopenia there is a need for a treatment regimen that can counteract the immobilization induced sarcopenia.

Growth hormone (GH) simulates postnatal growth, and exerts its effect either directly or indirectly by prompting the liver to produce insulin like growth factor I (IGF-I). GH is a potent anabolic agent known to promote skeletal muscle cell protein synthesis and growth [26]. Thus, rat studies have shown that GH can induce a substantial increase in muscle mass [27,28]. Consequently, GH is a viable candidate for mitigating the loss of muscle mass during disuse.

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https://doi.org/10.1016/j.bone.2018.02.017
8756-3282/

In unloaded rats, systemic treatment with intermittent PTH leads to increased bone formation on endosteal bone surfaces [29]. Furthermore, in ambulating rats intermittent PTH results in increased trabecular thickness, cortical bone thickness, bone density, and bone strength [30,31]. The bone anabolic effect of intermittent PTH is mediated by the G-protein-coupled PTH/PTHrP receptor. Upon agonist receptor interaction, the receptor can activate the cAMP-dependent protein kinase (PK)A and calcium-dependent PKC. Activation of PKC, however, account for most of the bone anabolic properties of intermittent PTH [32]. These involve direct effect on osteoblasts and indirect effects mediated by activation of skeletal growth factors such as IGF-I and inhibition of growth factor antagonists, such as sclerostin [32–34].

Taking the bone specific properties of PTH and the bone and muscle specific properties of GH into consideration, a combination treatment using these agents may counteract immobilization-induced osteopenia and sarcopenia and seems a rational therapeutic approach. The effect of PTH and GH combination treatment on the skeleton have previously been studied in ovariectomized [35–38] and hypophysectomized rats [39], whereas this treatment regime has not previously been investigated in disuse osteopenic animals.

Therefore, the aim of the present study was to investigate whether PTH and GH alone, or in combination, can prevent immobilization-induced osteopenia and sarcopenia.

2. Materials and methods

2.1. Animals and study design

The study comprised 72 female 12–14-week-old Wistar rats (Taconic, Ejby, Denmark). The rats were stratified according to their body weight (226.43 ± 1.79 g) into the following six groups with 12 rats in each group: 1. Baseline (Base); 2. Control (Ctrl); 3. BTX; 4. BTX + GH; 5. BTX + PTH; 6. BTX + PTH + GH. The animals were housed at 21 °C with 30% humidity, a 12/12 h light/dark cycle, and ad libitum access to tap water and food (Altromin 1324, Brogaarden, Lynge, Denmark). The animals in the Base group were euthanized at study start.

At the beginning of the study, the right hindlimb of the animals in the immobilization groups was injected i.m. with 4 IU BTX (Botox, Allergan, Irvine, CA, USA). BTX was administered as 2 IU into the quadriceps muscle, 1 IU into the hamstrings, and 1 IU into the posterior calf muscle of the right hindlimb [9]. The Ctrl group was injected with saline using the same regimen as the BTX injections. Human PTH (1–34) (H-4835, Bachem, Bubendorf, Switzerland) was dissolved in saline with 2% 56 °C heat inactivated rat serum and administered as s.c. injections (60 μg/kg/day) [38] 5 days a week. Biosynthetic human GH (Nodritropin, Novo Nordisk, Bagsværd, Denmark) was dissolved in sterile filtered phosphate-buffered saline and administrated as s.c. injections (5 mg/kg/day) twice daily [40] with at least 6 h between each injection 5 days a week.

The animals were injected s.c. with tetracycline (20 mg/kg, T3383, Sigma-Aldrich, St. Louis, MO, USA) 6 days before study start, calcein (5 mg/kg, C0875, Sigma-Aldrich, St. Louis, MO, USA) 4 and 5 weeks before euthanization, and alizarin (20 mg/kg, A3882, Sigma-Aldrich, St. Louis, MO, USA) 1 and 2 weeks before euthanization. This labeling regimen was applied in order to facilitate determination of bone formation in both the early and late phases of the experiment.

In order to assess the physiological effect of the BTX injections, the gait ability of 7 animals from each group was evaluated using the score by Warner et al. [41]. In brief, the gait ability score ranged from 0 (completely disabled) to 10 (normal) and was performed weekly starting at day 1 and lasted until the animals were euthanized. The treatment lasted for six weeks, and no animals died or were killed prematurely.

At the end of the experiment the animals were anesthetized with 3% isoflurane (IsoFlo Vet, Orion Pharma Animal Health, Nivå, Denmark) and killed with an overdose of 200 mg/kg pentobarbital i.p. (Mebumal, SAD, Copenhagen, Denmark). The rectus femoris muscles were isolated in a standardized manner. The right femora and tibiae were isolated and any soft tissue remaining was removed. Femora were stored in Ringer's solution at −29 °C, and tibiae were immersion-fixed in 0.1 M sodium phosphate buffered formaldehyde (4% formaldehyde, pH 7.0) for 48 h and then stored in 70% ethanol. The study complied with the guiding principles of the European Communities Council Directive of 24 November 1986 (86/609/EEC), and was approved by the Danish Animal Experiments Inspectorate (2012-15-2934-00769).

2.2. Rectus femoris muscles

Immediately after removal, the wet weight of the rectus femoris muscles was determined using a digital scale (Mettler AT250, Columbus, OH, USA). Rectus femoris muscles were immersion-fixed in 0.1 M sodium phosphate-buffered formaldehyde (4% formaldehyde, pH 7.0). Rectus femoris muscles were embedded in plastic based on 2-hydroxyethyl methacrylate (Technovit 7100; Heraeus Kulzer, Wehrheim, Germany), 2-μm-thick sections were cut on a microtome (Jung RM2065; Leica Instruments, Nussloch, Germany), and stained with Masson's trichrome.

The sections were scanned in a digital slide scanner (NanoZoomer-XR, Hamamatsu) and analyzed using newCAST (Version 6.4.1.2240, Visiopharm). Each section was sampled using systematic uniformly random sampling [42]. Using a 2D unbiased counting frame, striated muscle cell profiles within the counting frame not touching the exclusion lines were sampled. On average 189 muscle cell profiles were sampled for each animal. At a total magnification of ×1190 the cross sectional area (CSA) of the individual striated muscle cell profiles (muscle cell CSA) was estimated using the 2D nucleator principle [43]. In brief, the center of each muscle cell profile was marked, four intercept lines radiating from the center point were automatically drawn, and the intersection between the cell membrane and the intercept lines was marked interactively, and the CSA of each muscle cell profile was automatically estimated. The investigator was blinded for the group distribution during the evaluation.

The coefficient of variation of rat striated muscle cell profiles determined from digitalized sections is 8.2% in our laboratory (same sample measured 10 times).

2.3. Peripheral dual energy X-ray absorptiometry (pDXA)

Right femora were scanned in a pDXA scanner (Sabre XL, Nordland Stratec, Pfortzheim, Germany) at a scan speed of 10 mm/s and an isotropic pixel size of 0.5 mm and aBMD and BMC were automatically calculated.

Quality assurance was performed by daily scans of the two solid-state phantoms provided with the scanner. The coefficient of variation of rat femoral aBMD determined with pDXA is 2.8% in our laboratory (same sample measured 10 times).

2.4. Micro computed tomography (μCT)

The bones were scanned in a desktop μCT scanner (Scanco μCT 35, Scanco Medical AG, Brüttiselen, Switzerland). The distal femoral metaphysis and the femoral mid-diaphysis were scanned in high-resolution mode (1000 projections/180°), at an isotopic voxel size of 10 μm, an X-ray tube voltage of 70 kVp, a current of 114 μA, and an integration time of 800 ms. Beam hardening effects were reduced using a 0.5 mm aluminum filter.

The distal femoral metaphysis was analyzed using two separate 2200-μm-high volumes of interest (VOIs). The first VOI was demarked at the endosteal surface thus including trabecular bone only, while the second VOI was demarked at the periosteal surface including both trabecular and cortical bone, both starting 1500 μm below the most distal part of the growth zone in order to exclude primary spongiosa. The femoral mid-diaphysis was analyzed using a 2300-μm-high VOI including