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Ibandronate treatment reverses glucocorticoid-induced loss of bone mineral density and strength in minipigs

C.C. Glüer [a,*], K.E. Scholz-Ahrens [b], A. Helfenstein [c], G. Delling [d], W. Timm [e], Y. Açil [f], R. Barkmann [a], J. Hassenpflug [c], B. Stampa [e], F. Bauss [g], J. Schrezenmeir [b]

[a] *Medical Physics, Department of Diagnostic Radiology, University Hospital Schleswig-Holstein, Campus Kiel, Germany*
[b] *Institute of Physiology and Biochemistry of Nutrition, Federal Research Centre for Nutrition and Food, Location Kiel, Germany*
[c] *Department of Orthopedic Surgery, University Hospital Schleswig-Holstein, Campus Kiel, Germany*
[d] *Institute of Bone Pathology, University Hospital Hamburg-Eppendorf, Hamburg, Germany*
[e] *Synarc A/S, Hamburg, Germany*
[f] *Department of Oral and Maxillofacial Surgery, University Hospital Schleswig-Holstein, Campus Kiel, Germany*
[g] *Roche Diagnostics GmbH, Pharma Research Penzberg, Penzberg, Germany*

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Abstract

The Göttingen minipig is one of the few large animal models that show glucocorticoid (GC)-induced bone loss. We investigated whether GC-induced loss of bone mineral density (BMD) and bone strength in minipigs can be recovered by treatment with the bisphosphonate ibandronate (IBN).

40 primiparous sows were allocated to 4 groups when they were 30 months old: GC treatment for 8 months (GC8), for 15 months (GC15), GC treatment for 15 months plus IBN treatment for months 8–15 (GC&IBN), and a control group without GC treatment. Prednisolone was given at a daily oral dose of 1 mg/kg body weight for 8 weeks and thereafter 0.5 mg/kg body weight. IBN was administered intramuscularly and intermittently with an integral dose of 2.0 mg/kg body weight. BMD of the lumbar spine (L1–3) was assessed *in vivo* by Quantitative Computed Tomography (QCT) at months 0, 8, and 15. Blood and urine samples were obtained every 2–3 months. After sacrificing the animals lumbar vertebrae L4 were tested mechanically (Young's modulus and ultimate stress). Histomorphometry was performed on L2 and mineral content determined in ashed specimens of T12 and L4.

In the GC&IBN group, the GC associated losses in BMD of −10.5%±1.9% (mean±standard error of the mean, $p<0.001$) during the first 8 months were more than recovered during the following 7 months of IBN treatment (+14.8%±1.2%, $p<0.0001$). This increase was significantly larger ($p<0.0001$) than the insignificant +2.1%±1.2% change in group GC15. At month 15, the difference between groups GC&IBN and GC15 was 22% ($p<0.01$) for BMD, 48% ($p<0.05$) for Young's modulus, and 31% ($p<0.14$) for ultimate stress; bone-specific alkaline phosphatase showed trends to lower values ($p<0.2$) while deoxypyridinoline was comparable.

This minipig study demonstrates that GC-induced impairment of bone strength can be effectively and consistently treated by IBN. GC&IBN associated alterations in BMD and bone turnover markers can be monitored *in vivo* using QCT of the spine and by biochemical analyses, reflecting the changes in bone strength.

Keywords: Glucocorticoid; Bisphosphonate; Minipig; Bone strength; Bone densitometry

Introduction

Glucocorticoid (GC)-induced bone loss and the increased risk for fractures represents one of the most severe forms of secondary osteoporosis. Particularly in patients beginning an oral GC treatment (due to e.g. rheumatoid arthritis, chronic

* Corresponding author. Medizinische Physik, Klinik für Diagnostische Radiologie, Universitätsklinikum Schleswig-Holstein, Campus Kiel, Michaelisstr. 9, D-24105 Kiel, Germany. Fax: +49 431 597 3127.
E-mail address: glueer@rad.uni-kiel.de (C.C. Glüer).

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obstructive lung disease, Crohn's disease, or transplantation), bone loss as measured by Dual-X-ray Absorptiometry (DXA) is substantial and is greatest in the first 3 to 6 months, depending on the dose and the underlying disorder [47,48]. Bone loss in the order of about 30% of trabecular bone mass has been reported to occur already within 6 months [38] and slows down over prolonged treatment periods. The problem is enhanced further because several authors have reported that the associated increase in fracture risk even surpasses the effect expected from this magnitude of loss in bone mineral density (BMD) [65]. Thus GC-induced osteoporosis (GIO) is a condition that severely affects patients' health and quality of life which in consequence had led several societies (e.g., the American College of Rheumatology in the United States [1] and the Dachverband Osteologie in Germany [40]) to require bone densitometry testing in subjects initiating GC treatment [19].

Fortunately, some of the treatments developed for primary osteoporosis are also effective in GIO. GC-induced bone loss can be effectively treated by bisphosphonates. In a meta-analyses, in which 45 clinical studies with 49 eligible treatment comparisons were investigated, bisphosphonates were found to be the most effective class for managing corticoid-induced osteoporosis [5]. For example, in a randomized-controlled trial on 290 men and women on oral GC treatment exceeding 7.5 mg/day prednisone or equivalent a significant improvement in BMD compared to the placebo group was achieved and the incidence of vertebral fractures could be reduced by 70% ($p<0.05$) in patients on oral daily risedronate treatment [46]. For residronate, a cyclical regimen was equally effective for protecting against GC-induced BMD loss at the femur but was less efficacious at the spine when compared to daily administration [23]. Besides risedronate [46,67], the efficacy of oral bisphosphonate treatment of GC-induced osteoporosis has also been established for etidronate [3], and alendronate [4,50]. Improvements of BMD and reductions in vertebral fracture risk have been reported for intermittent (3 months) intravenous ibandronate (IBN) injections [49].

The pathophysiological processes that lead to GIO have only been partially elucidated. Currently accepted underlying mechanisms include suppressed bone formation due to decreased activity and shortened lifespan of osteoblasts, intestinal malabsorption of and reduced renal reabsorption of calcium, which leads to secondary hyperparathyroidism, reduced osteocyte lifespan, and promotion of osteoclast survival [69,70]. The histomorphometric bone features are decreases in trabecular bone mass, trabecular width, osteoid area, osteoid width, osteoid density, osteoblast density, mineralizing surface, and mineral apposition rate [69].

One obstacle that hinders more rapid progress in this area is the paucity of established animal models. The effects of GC on bone are complex and differ between species. In rats unlike in humans, bone resorption was reported to be inhibited leading to increased bone mass [34,59]. The choice of the measurement site may be of importance [68]. Data on large animal models are scarce. In growing piglets, GC reduced skeletal growth and BMD gain already within 12 days [24] while in adult beagle dogs a 13% bone loss was observed after 48 weeks [39]. GC-induced bone loss due to inhibited bone formation has been reported in sheep [17,18,35,36,57] but in some studies BMD and strength reductions could not be observed and other authors reported site-specific differences in the impact on bone strength [57].

We have reported that the minipig is a good large animal model for GC associated bone loss [56] demonstrating a vertebral bone loss measured by Quantitative Computed Tomography (QCT) of $-13.6\% \pm 1.9\%$ in the first 8 months of GC treatment ($p<0.0005$) with stable BMD levels thereafter and stable BMD also for the control group [26]. The histomorphometric analysis of trabecular bone of lumbar vertebrae confirmed the effects showing a significant loss of bone volume per tissue volume ($p<0.05$) and a trend to decreased connectivity ($p<0.1$) [56].

In the present study, we report the effects of the bisphosphonate ibandronate on the skeletal response of GC-treated minipigs. The goal of our study was to investigate whether the reported GC-induced bone loss could be restored by bisphosphonate treatment and whether lost bone strength could be regained. In order to characterize the pathophysiological processes and, thereby, further study the value of the minipig as a large animal model for treatment of GC-induced bone loss, a variety of different diagnostic methods were employed, including bone densitometry, biochemical markers of bone turnover, histomorphometry, and biomechanical testing.

Materials and methods

Animals

As part of a larger study, 40 primiparous adult female Göttingen minipigs of the Institute of Physiology and Biochemistry of Nutrition's breeding herd were included in this investigation at the age of 30 months. In order to standardize the lactation productivity, the litter size was set to five to six piglets and the nursing time to 6 weeks. The experiment was approved by the responsible governmental review board for animal studies ("Ministerium für Umwelt, Natur und Forsten des Landes Schleswig-Holstein").

Housing and diets

After the nursing time, animals were transferred to individual housing for the whole experimental time. The sows were kept strawless on floor cages. At the same time the sows were switched from the Institute's standard nutrition regimen for lactating minipigs to the Institute's semi-purified diets for adult minipigs. This diet contained mineral and vitamin pre-mixtures to provide all nutrients necessary for minipigs in sufficient amounts but was otherwise adapted to an average human diet (rich in fat and protein, poor in fiber). Feed intake was controlled individually and was restricted to 370 g/day in order to allow a steady and slow growth as it is typical for this age, avoiding fattening. Deionized water was available ad libitum.

Treatment and animal grouping

After an adaptation time of 4 weeks (pre-experimental) to these study conditions the sows were allocated to one of four experimental groups on the basis of their body weights. Except for animals in the control group (CNTR) all animals received GC treatment during the first 8 months of the study. At month 8, the animals that had been allocated to the short term GC treatment group (GC8) were sacrificed. The other animals continued GC treatment for another 7 months, half of them allocated to continued GC treatment (GC15) while the