Parathyroid hormone (1-34) prevents cartilage degradation and preserves subchondral bone micro-architecture in guinea pigs with spontaneous osteoarthritis

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Objective: To assess whether parathyroid hormone (PTH) (1-34) could improve the micro-structure of subchondral bone, and retard cartilage degradation in a naturally occurring Osteoarthritis (OA) model.

Design: Forty-eight 1-month-old guinea pigs were divided into two groups: 32 were treated by normal saline (NS) and sacrificed at 1, 3, 6 and 9 months of age; the other 16 received PTH (1-34) from 3 months, and were sacrificed at 6 and 9 months. Masson staining and the Osteoarthritis Research Society International (OARSI) grade scores were used to assess cartilage degradation. Immunohistochemistry analyses of type-II collagen, matrix metalloproteinases-13 (MMP-13) and sclerostin (SOST) in the cartilage, osteoprotegerin (OPG) and receptor activator of nuclear factor-kB ligand (RANKL) and PTH receptor (PTH1R) in the cartilage and subchondral bone were performed. Subchondral bone micro-architecture was assessed by micro-computed tomography (micro-CT).

Results: Histological analyses revealed OA occurred at 3 months of age and was more severe with increasing age, and PTH (1-34) reduced the OARSI scores at 6 and 9 months of age. Micro-CT analysis indicated that PTH (1-34) treatment increased the bone volume ratio and bone mineral density (BMD), while retarding the subchondral trabecular bone micro-architectural changes from rod-like to plate-like. Immunohistochemical staining demonstrated that PTH (1-34) treatment increased type-II collagen expression and decreased SOST and MMP-13 expression in the cartilage, while elevating the PTH1R, OPG/RANKL expression ratio in the cartilage and subchondral trabecular bone when compared with the control groups.

Conclusions: PTH (1-34) can prevent cartilage damage progression and retard the deterioration of subchondral trabecular bone in guinea pigs.

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Introduction

Osteoarthritis (OA) is one of the most prevalent joint disorders, which has brought enormous challenges to social economics. OA has been recently redefined as a disease that affects the whole joint, including the articular cartilage and subchondral bone.

Although it is unclear whether precedes or follows cartilage damage, subchondral bone degeneration is an important feature of the OA physiopathology. There is increasing evidence to support the concept that skeletal adaptations antedate detectable alterations in the structural integrity of the articular cartilage. These alterations include a progressive increase in subchondral plate thickness, changes in the subchondral trabecular bone architecture, formation of new bone at the joint margins (osteophytes), and development of subchondral bone cysts. Skeletal changes in OA may be associated with the differentiative adaptive capacity of...
cartilage and subchondral bone to adapt to mechanical loading and damage. Because of this, optimal treatment options for OA should entail both bone and cartilage protective effects.

Parathyroid hormone (PTH) acts as the most important regulator of calcium homeostasis in the human body through its direct action on bone and the kidneys. Previous studies have shown that intermittent administration of certain analogs of PTH produces an anabolic effect on bone. Intriguingly, a new pharmacological characteristic of PTH suggests it may have a possible therapeutic benefit in the treatment of OA. A previous study has revealed that PTH (1-34) acted on human articular chondrocytes to suppress their terminal differentiation and also suppressed papain-induced OA in rats. Further, recent research using a rabbit osteochondral defects model, indicates that PTH (1-34) improves articular cartilage surface architecture and integration, and subchondral bone reconstitution. However, to date, there have been no reports about the effect of PTH (1-34) on primary OA.

Studies about primary OA progression in humans are difficult because of slow disease progression, and symptoms are often only present in the later stages of the disease. As a consequence, numerous animal models have been developed, but most are second order to models using mechanical instability or chemical intervention to drive the disease, which are not ideal models for researching primary OA. Dunkin Hartley (DH) strain guinea pigs are a well established and widely used naturally occurring OA model. Importantly, the appearance of joint pathology in both guinea pigs and humans is age-related; guinea pigs are subject to a variety of well-recognized OA risk factors, which are shared with humans.

We therefore hypothesized that the bone-forming agent PTH (1-34) may have therapeutic potential for treating OA. The present study was designed to test this hypothesis by detecting the cartilage degeneration, extracellular matrix metabolism, and micro-architecture changes in the subchondral bones of DH guinea pigs with primary OA.

Materials and methods

Animal handling

All experiments were approved by Hebei United University Animal Care and Use Committee. Forty-eight 1-month-old female Dunkin Hartley guinea pigs (Vital River Experimental Animal Technical Co., Ltd, Beijing, China) were fed a standard rodent diet and housed in the Medical Research Animal Center. They were randomly assigned into two groups as follows: 32 were treated by PTH (1-34) (Sigma Chemical Co., USA) at a dosage of 15 μg/kg/day for 5 days per week as control group, and sacrificed at 1, 3, 6 and 9 months of age (eight animals at each time point); the other 16 received subcutaneous injections of PTH (1-34) (Sigma Chemical Co., USA) at a dosage of 15 μg/kg/day for 5 days per week from 3 months of age, and were then sacrificed at 6 and 9 months of age (eight animals at each time point). The body weight of every animal in each group was recorded.

Gross observations and specimen processing

After disarticulation, both femurs and tibias were cleaned and their gross visual appearance recorded with a digital camera (Canon 550D, Canon, Japan). The femurs were harvested for paraffin sections; immunohistochemistry was conducted once femurs were stained with Masson. The harvested tibias were fixed in 70% ethanol for subchondral micro-computerized topographic (micro-CT) testing.

Tissue preparation and cartilage histopathological analysis

All femurs were fixed in 4% paraformaldehyde for 48 h and then decalcified with 15% EDTA-Na2 (pH 7.4) at 4°C for 6 weeks. The distal end of femurs were dehydrated, embedded in paraffin, and cut into 5-μm thick sections (according to standard protocols). Four sections from each sample were stained with Masson. Then, three color digital images from each section were recorded under light microscopy (Olympus BX61, Olympus, Japan). Semi-quantitative histopathological analysis was established according to the Osteoarthritis Research Society International (OARSI) score system using five characteristics: articular cartilage structure, proteoglycan content, cellularity, tidemark integrity, and osteophytes. For each animal the scoring was performed by a blinded observer using two non-consecutive Masson stained sections.

Immunohistochemistry for type-II collagen, matrix metalloproteinases-13 (MMP-13), sclerostin (SOST), PTH receptor (PTH1R), osteoprotegerin (OPG) and receptor activator of nuclear factor-kB ligand (RANKL)

To further clarify the cartilage degeneration and subchondral bone changes, type-II Collagen, MMP-13 and SOST expression in the cartilage, PTH1R, OPG and RANKL expression in the cartilage and subchondral bone were detected by way of immunohistochemistry. Tissue sections were routinely deparaffinized, rehydrated and repaired in complex phosphoesteraseum for 10 min at 37°C, and then incubated overnight at 4°C with anti-rabbit type-II collagen (1:200), MMP-13 (1:150), OPG (1:100) and RANKL (1:100) (Boster Corporation, Wuhan, China). The rest procedures were manipulated according to the PV-6001 Two-Step IHC Detection Reagent illustration (ZSGB-BIO Corporation, China); the color brown was developed using DAB (ZSGB-BIO Corporation). The sections were counterstained with hematoxylin. The sample appearing yellow or brownish yellow was considered as positive staining. All sections were semi-quantitatively analyzed by Image Pro Plus (IPP) version 6.0 software, and the integrated optical density (IOD) was measured by the staining in six fields in each section on the images at 400× magnification, the average IOD from three observers was the final observation result and used for statistical analysis.

Subchondral bone microstructural measurement

The proximal tibiae was scanned using a high-resolution micro-CT system (SkyScan, Aartselaar, Belgium) to quantify the subchondral bone plate thickness (defined as starting from the calcified cartilage-bone junction and ending at the marrow space) and micro-architecture of the subchondral trabecular bone, which was defined as the epiphyseal cancelous bone region 0.5 mm beneath the subchondral plate of medial tibiae, with voxel size 9 μm, the energy and intensity were equal to 40 kVp and 250 μA, respectively. The binary images were reconstructed to 3D for qualitative and quantitative evaluations. The following 3D morphometric parameters were calculated to describe the bone mass and structure: bone mineral density (BMD), bone volume ratio (BV/TV), trabecular thickness (Tb.Th), and the Structure Model Index (SMI).

Statistical analysis

All data are expressed as the mean ± SD. Comparisons between different age control groups were tested using a one way analysis of variance (ANOVA) and Fisher's least significant difference (LSD) t-test. Direct effects from treatment with PTH (1-34) were assessed using an independent t test between PTH-treated and NS-treated