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Parathyroid hormone(1-34) exhibits more comprehensive effects than celecoxib in cartilage metabolism and maintaining subchondral bone micro-architecture in meniscectomized guinea pigs

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SUMMARY

Objective: To evaluate the effects of PTH(1-34) on cartilage, subchondral bone mass and structure in medial meniscectomized guinea pigs and compare them to those of celecoxib (CLX).
Method: Forty-eight 3-month-old male Hartley albino guinea pigs received either sham or medial meniscectomy (MNX) operations. One week after the procedure, meniscectomized animals began 12 weeks of treatment by oral administration of CLX (20 mg/kg, daily), subcutaneous injection of PTH (1-34) (24 μg/kg, 5 days/week), or normal saline for MNX group. All animals were euthanized 12 weeks later, cartilage degeneration and subchondral bone micro-architecture was analyzed.
Results: OARSI scores indicated cartilage degeneration was partially inhibited by either CLX or PTH(1-34). Cartilage was significantly thicker in PTH(1-34)-treated animals than in CLX-treated animals. Both CLX and PTH(1-34) treatment were associated with lower ADAMTS-4 and periostin expression than MNX. MMP-13 expression in PTH(1-34) group was significantly lower than that in CLX group. However, AGG expression and the ratio of Col-II/MMP-13 expression in PTH(1-34) group were significantly higher than in the CLX group. Micro-CT analysis showed BMD, BV/TV, and Tb.Th levels to be significantly lower in the MNX group and CLX groups than in the sham group, but these parameters were significantly higher in the PTH(1-34) group than in either the MNX group or CLX group.
Conclusions: Both CLX and PTH(1-34) exhibits protective effects on cartilage degeneration in meniscectomized guinea pigs. However, PTH(1-34) exhibited superior performance to CLX not only in metabolism of cartilage tissue but also in maintenance of subchondral bone micro-architecture.

Introduction

Osteoarthritis (OA) is a common type of degenerative disease troubling the elderly. Currently, there is no pharmaceutical therapy that can retard the progression of OA. PTH(1-34) is currently used for treatment of osteoporosis. It has been found that PTH(1-34) not only helps maintain bone mass but also helps chondrocytes promote cell proliferation and extracellular matrix (ECM) production[1–4]. PTH(1-34) may have disease-modifying effects, and its effects have been confirmed by a recent study in a post-trauma OA mouse model[5]. *In vitro* studies have indicated that the beneficial effects of PTH(1-34) on chondrocytes involve binding to parathyroid hormone/parathyroid hormone-related peptide receptor (PTH1R) through activated multiple pathways[6–8]. In addition to the direct effects on chondrocytes, there has been a study in an OA model indicating that PTH(1-34) may also reduce synoviopathy[9]. There have also been studies showing that the improvement of subchondral bone integrity in response to PTH (1-34) contributes to the reduction of cartilage damage progression in animal models[10,11]. These results suggest that PTH(1-34) may have multiple effects on the entire affected joint.

Celecoxib (CLX) is recommended as an option for pharmaceutical therapy in the OA treatment guidelines of the American

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Academy of Orthopaedic Surgeons (AAOS)[12]. Although CLX can effectively relieve stiffness and pain in joints, due to its anti-inflammatory effect by inhibiting cyclooxygenase-2 (COX-2)[13]. It has little effect on joint space narrowing in humans[14]. However, animal experiments have shown that CLX still has effects in mitigation of joint degeneration by reducing osteophyte development[15]. This could be explained by a previous study showing that CLX could decrease RANKL synthesis and subsequently increase the OPG/RANKL ratio in the cartilage which may further affect subchondral bone metabolism[16]. *In vitro* studies have also demonstrated that its effects may involve repressing chondrocyte apoptosis[17,18].

It seems possible that PTH(1-34) may be more effective than CLX in the treatment of OA. Because there has been little study of the effects of PTH(1-34) in human OA so far, the aim of the present study was to compare the effects of PTH(1-34) to those of CLX in medial meniscectomized (MNX) guinea pigs, which here serve as an animal model of the early progression of OA.

Methods

Animal handling

Forty-eight 3-month-old male SPF (specific-pathogen free) grade Dunkin Hartley (DH) albino guinea pigs (Vital River Experimental Animal Technical Co., Ltd., China) were housed in pairs and given 1 week to acclimate to the housing facility. Environmental conditions included a temperature of 25°C ± 1°C, humidity 55% ± 10%, and a 12:12 light: dark cycle with lights on at 07:00 and off at 19:00. Animals were housed in 545 × 380 × 200 mm cages and given access to a sterilized diet (60Co Guinea Pig Diet 3035, Beijing HFK Bioscience Co., Ltd., China) and water *ad libitum*. Environmental enrichment was four handfuls of sterilized sawdust nesting material. During housing, animals were monitored once daily for health status and environmental maintenance. No adverse events were observed. At the start of the experiments, animals weighed 747 ± 58 g (mean ± SD). Animals were randomly divided into four groups as follows: sham group, MNX, CLX, and PTH groups, twelve animals per group. Animals were anesthetized by intraperitoneal injection of sodium pentobarbital (30 mg/kg), medial meniscectomy (MNX) were performed for MNX, CLX, and PTH groups according to the protocol described by Bendele[19]. A sham operation consisting of only an incision in the skin at the same location was performed on animals in the sham group. Animals were all carefully handled and kept warm during and after surgery. None of the surgeries failed and no animals died during the experiment.

Drug administration was initiated 1 week post-surgery. Animals in CLX group received celebrex (Pfizer, Inc., US) by oral gavage at a dosage of 20 mg/kg daily. Animals in PTH group received PTH (1-34) (Sigma–Aldrich Corp., US) subcutaneous injection 5 days per week at a dose of 24 μg/kg. Saline was given as placebo to animals in MNX group. Each group was then randomly divided into two subgroups containing equal numbers of guinea pigs for further macroscopic scoring/micro CT testing ($n = 6$) or histological scoring/immunohistochemistry assays ($n = 6$). Animal treatment lasted for 12 weeks. The animals' body weight showed no statistical significant difference between groups at the end point of treatment. All animals were euthanized by intraperitoneal overdose injection of sodium pentobarbital to harvest blood and operated knee joints by the end of treatment. All reasonable efforts were made to minimize pain and suffering. All experiments were approved by the university's Animal Care and Use Committee.

Specimen processing and OARSI scoring

The primary outcomes were defined using Osteoarthritis Research Society International (OARSI) scoring results, and other results were considered secondary outcomes. All samples for macroscopic scoring were sequentially disarticulated and cleaned and their gross visual appearances were recorded using a digital camera (Canon 550D, Canon, Japan). Specimens were then fixed in 100% ethanol for micro-Computed Tomographic (micro-CT) assessment. The gross visual documents were evaluated for the stage of the disease according to OARSI macroscopic scoring system in a blinded fashion[20].

All samples used for histological assessment were directly fixed in 4% neutral formalin solution for 48 h and then decalcified with 15% EDTA-Na_2 (pH 7.25) at 4°C for 6 weeks. The samples were sequentially dehydrated, embedded in paraffin, and cut into 8 μm sections. Three non-consecutive coronal sections from each sample were stained with toluidine blue. Then, one digital image for each section was recorded under an optical microscope (Olympus BX53, Olympus, Japan). Semi-quantitative histopathological analysis was established according to the OARSI microscopic scoring system[20]. The thickness of the cartilage of the medial tibial plateau was measured further on these sections. The scoring and measurement processes were also performed in blinded fashion and the average of the three sections served as the datum for that sample.

Subchondral bone microstructure measurement

The proximal tibiae were scanned using a micro-CT system (ZKKS-Sharp-MCT, Guangzhou, China) to quantify micro-architecture of subchondral trabecular bone, and the region of interest (ROI) was defined as the epiphyseal cancellous bone region 0.5 mm beneath the subchondral plate of medial tibiae, with voxel size of 20 μm. The energy and intensity were equal to 40 kVp and 250 mA, respectively. The following morphometric parameters were calculated using software developed for the machine to describe the bone mass and structure: bone mineral density (BMD), bone volume ratio (BV/TV), trabecular number (Tb.N), trabecular spacing (Tb.Sp), trabecular thickness (Tb.Th), trabecular bone pattern factor (TbPf), structure model index (SMI), and degree of anisotropy (DA).

Immunohistochemical assessment

For further investigation of cartilage status, Aggrecan (AGG), collagen-II (Col-II), caspase-3, a disintegrin and metalloproteinase with thrombospondin motifs 4 (ADAMTS-4), metalloproteinase-13 (MMP-13), and periostin were detected using immunohistochemistry. Paraffin sections were deparaffinized, rehydrated, and subjected to routine antigen retrieval manner using 0.05% trypsin at 37°C for 30 min, and endogenous peroxidase activity was suppressed by 0.3% H_2O_2 for 15 min, then incubated overnight at 4°C with the following antibodies: AGG (1:200) (PAB908Ra02, Cloud-Clone Corp., US), ADAMTS-4 (1:200) (ab185722, Abcam Inc., US), Caspase-3 (1:200) (PAA626Ra01, Cloud-Clone Corp., US), Col-II (1:50) (II-II6B3 was deposited to the DSHB by Linsenmayer, T.F.), MMP-13 (1:200) (PAA099Ra01, Cloud-Clone Corp., US), and periostin (1:200) (PAH339Ra01, Cloud-Clone Corp., US), respectively. Other procedures were performed according to the instructions provided with the PV-6001 Polink-1 HRP DAB Detection System (ZSGB-BIO Corp., China) and ZLI-9017 DAB kit (ZSGB-BIO Corp., China). The sections were counterstained with hematoxylin. The level of expression of target protein in cartilage tissue of the tibial plateau was evaluated using the average intensity of optical density. The average intensity of optical density, given in IOD/mm^2, was

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