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Effect of calcitonin in early and late stages of experimentally induced osteoarthritis. A histomorphometric study

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Summary

Objective: To investigate both prophylactic and therapeutic roles of salmon calcitonin on the articular cartilage of rabbit's knees.

Methods: Right knee instability was produced in 30 New Zealand white rabbits by sectioning the cranial cruciate ligament (CCL). Animals were separated into four groups: placebo prophylactic-stage group ($n=6$), killed 8 weeks post surgery, calcitonin prophylactic-stage group ($n=6$), treated immediately after surgery with salmon calcitonin and killed at 8 weeks, placebo therapeutic-stage group ($n=9$) killed at 16 weeks post surgery and calcitonin therapeutic-stage group ($n=9$), treated with salmon calcitonin from 8th to 16th week and killed at 16 weeks post surgery. A histomorphometric study was based on the morphological changes of the articular cartilage and subchondral bone (degeneration indexes), as well as the articular cartilage thickness, chondrocytes' arrangement and their metabolic activity (regeneration indexes).

Results: Calcitonin groups showed smoother articular surface, no or minimal signs of ulceration, smaller osteophytes, and less subchondral cystic formation than placebo groups. Normal distribution of chondrocytes or hypercellularity was noticed in areas of mild osteoarthritic (OA) changes in the calcitonin groups indicating regeneration activity. Periodic Acid Schiff's and Alcian blue staining were negative in the placebo groups while increased absorption in the calcitonin groups revealed high anabolic activity.

Conclusions: In prophylactic stages salmon calcitonin seemed to inhibit the progression of osteoarthritis by increasing the layers of hyaline cartilage, restoring the cellular metabolism, and decreasing the volume of osteophytes. In therapeutic stages, the hormone had a healing effect by decreasing the subchondral cysts, regenerating the hyaline cartilage and restoring cellular metabolism. Both macroscopic and histological findings of this study supported the biochemical results of previous studies showing the therapeutic effect of calcitonin on osteoarthritis.

Key words: Calcitonin, Osteoarthritis, Animal studies, Anterior cruciate ligament Transection, Histology.

Introduction

Conservative treatment regimens for osteoarthritis treat the symptoms but not the disease and are limited to control pain and inflammation and eliminate the risk factors[1,2]. Furthermore, it is still debated if simple analgesics are effective as oral drugs of first choice[3,4]. Steroidal and non-steroidal anti-inflammatory drugs (NSAIDs) are associated with serious adverse events particularly gastrointestinal. Selective cyclo-oxygenase-2 (COX-2) inhibitors reduce the incidence of upper gastro-intestinal tract ulcerations, however, other toxicities such as fluid retention, hypertension, congestive heart failure, renal insufficiency and a risk for cardiovascular thrombosis may occur[5,6]. The therapeutic role of high molecular weight hyaluronans, chondroitin sulfate and glucosamine in terms of pain relief and slowing the progression of osteoarthritis is also debated[7–9]. Finally, arthroplasty for end stage disease process has a limited life span, relieves pain more predictably than it improves joint function and can be associated with local and systematic complications[10].

Seeking other pharmaceutical interventions, calcitonin may be a potential agent for the treatment of osteoarthritis. Previous data indicated calcitonin's safety and effectiveness on reduction of bone turnover[11] and it's analgesic effect in relieving osteoarthritic (OA) pain[12,13]. Furthermore, there is *in vivo* and *in vitro* experimental evidence that calcitonin acts on both cartilage and subchondral bone by decreasing the enhanced turnover of the OA subchondral bone, reducing the severity of cartilage OA lesions and altering the biochemical composition and supramolecular organization of the OA cartilage matrix[14,15].

The purpose of this study was to evaluate macroscopically and microscopically the effect of salmon calcitonin on the articular cartilage in prophylactic and therapeutic stages of experimentally induced OA in rabbits and to determine the preventive and/or reparative activity of the hormone.

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Materials and methods

The experimental protocol was approved by the Veterinary Directorate (permit no.1130), according to the Greek Presidential Decree 160/1991, which conforms to the EEC Directive 609/1986 for the "protection of vertebrate animals used for experimental or other scientific purposes". Thirty adult male New Zealand white rabbits (*Oryctolagus cuniculus*), conventionally bred, were used. The animals were obtained from the conventional breeding facilities of the Hellenic Pasteur Institute and housed singly in steel cages of 45 cm × 30 cm × 60 cm dimensions (IFFA CREDO, BP 0109-69592, L'Arbresle, France) in the Laboratory for Research of the Musculoskeletal System conventional animal house. Their age ranged from 4 to 6 months and their weight ranged from 3.4 to 4.9 kg. The age and X-rays determined that the animals were skeletally mature. Additionally, the animals were randomly assigned so that each group had 4, 5 and 6 months old animals in them. The temperature therein ranged between 18 and 21°C, relative humidity 50–60%, the light/dark cycle was from 06:00 to 18:00 and there were 15 air changes/hour. The animals' body weight was 2040 ± 200 g (mean ± SD). They had free access to standard rabbit pellets (14% protein, 7% fat, 15% cellulose, 1–1.2% calcium and phosphorus) (ELVIZ Hellenic Feedstuffs Ind. SA, Plati, Imathia, Greece) and tap water.

SURGERY

All animals were operated on day 1. After induction of general anesthesia (ketamine 25 mg/kg and midazolam 5 mg/kg intramuscularly), the right stifle was shaved and the skin prepped. Anesthetic depth was monitored throughout the procedure by a DINAMAP vital signs monitor 1846. Through a medial parapatellar incision the cranial cruciate ligament (CCL) was transected macroscopically with a No 11 blade. After transection, anterior instability was manually confirmed by the anterior drawer test. The incision did not disrupt the patellar apparatus and no patellar luxation was needed for the CCL transection. The articular capsule and the medial retinaculum were closed with absorbable sutures and the skin with nylon sutures. After recovery, rabbits received paracetamol suppository (Dolal supp. bebe, 1/3 supp. = 50 mg) for pain relief and were free to move in their cages without any external immobilization. They also received paracetamol syrup (Dolal sir. 1 ml = 25 mg) twice a day the next postoperative day. The animals were checked daily (activity, body weight, food consumption, rectal temperature, wound healing) for signs of ill health for the first postoperative week.

TREATMENT REGIMENS

The animals were separated into four groups depending on the time of euthanasia and the treatment received. The first group, *placebo prophylactic-stage group* included six rabbits that were euthanised at 8 weeks in order to confirm the extension and grade of the developed OA lesions. In the literature, the period between 6 and 8 weeks after sectioning of the CCL is considered sufficient for the development of osteoarthritis in rabbits[16]. The second group, *calcitonin prophylactic-stage group* included six rabbits that received 7 IU salmon calcitonin injected intramuscularly daily from day 1 to week 8 postoperatively and then killed. The third group, *placebo therapeutic-stage group*, included nine rabbits that were placebo treated and euthanised at the sixteenth postoperative week. The fourth group, *calcitonin therapeutic-stage group*, included nine rabbits that received 7 IU salmon calcitonin injected intramuscularly daily from week 8 to week 16 postoperatively and then killed. Euthanasia was carried out by ketamine/midazolam premedication administered intramuscularly, followed by slow intravenous administration of sodium thiopental (20–30 mg/kg until cessation of cardiac function) in normal saline drip.

SPECIMEN COLLECTION

After euthanasia, all specimens were prepared as follows: excision of the skin, osteotomy 3 cm above and below the knee joint and fixation of the specimens in 10% buffered formalin for 24 h. Fixed specimens were cleared from soft tissues and ligaments, allowing the gross examination of the articular surfaces of the femoral condyles and tibial plateaus and charting of the specimens. The specimens were then decalcified in 10% nitric acid for 3 to 5 days. A non-operated control group was not employed for two reasons: (1) no OA or degenerative changes were observed in any of the joints during surgery and (2) rabbits have very little spontaneous degeneration in their knee joints[17].

SPECIMEN EXAMINATION

The macroscopic OA lesions included (1) osteophytes, (2) cartilage erosion (ulceration, fissures) and (3) loss of cartilage luster (softening, fibrillation). The evaluation of these lesions was based on three macroscopic parameters: (1) the location, (2) the type, and (3) the size of the OA changes. Macroscopic evaluation of the specimens included: (1) articular surfaces digital photographing (Nikon F5, Nikon Co, Japan) (×4 objective zoom) and scanning of the negative films (Scanjet 3200C, Hewlett Packard, Palo Alto, CA, USA), (2) the articular changes of the femoral condyles, femoral trochlea, and tibial plateaus were drawn on millimeter paper (×2 magnification), and (3) imprint on carbon paper of the OA changes with the use of ink paper. The area of the articular surface involving these lesions was measured using a Vernier caliper. The articular surfaces of the knee were divided in five sections (I–V) in order to define the areas with the highest incidence of OA alterations and standardize the size of histological sections (Table I).

Table I
Location of the macroscopically observed osteoarthritic (OA) changes in all groups

Site	Articular surface area	Predominant OA lesions
I	Femoral trochlea	Osteophytes
II	Middle of the medial femoral condyle	Severe ulceration and loss of luster
III	Middle of the lateral femoral condyle	Modest ulceration and loss of luster
IV	Anterior part of the medial and lateral tibial plateau	Mild ulceration and loss of luster
V	Posterior part of the medial and lateral tibial plateau	Mild ulceration and loss of luster