Oral calcitonin in the management of osteoarthritis: hope or fantasy?

In the mid-1980s, calcitonin was used as a potential treatment for postmenopausal osteoporosis. However, after the results obtained in a pivotal study assessing the antifracture efficacy of the drug showed an absence of reduction in nonvertebral fractures, calcitonin has almost completely disappeared from the osteoporosis armomentarium. The development of a new 'high-tech' oral formulation of salmon calcitonin and the demonstration, in several *in vitro* and *in vivo* models of osteoarthritis, that this drug could exert beneficial effects on the chondrocytes and on the development of experimental osteoarthritis has generated some interest in this old molecule. However, at this stage, results from clinical trials remain inconclusive and caution should be exerted before considering oral calcitonin as a breakthrough in the management of osteoarthritis.

KEYWORDS: calcitonin ■ cartilage ■ osteoarthritis ■ treatment ■ type II collagen

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Osteoarthritis (OA) is a progressive disorder characterized by destruction of articular cartilage and subchondral bone, and is associated with synovial changes [1,2]. This degenerative condition affects aging men and women [3]. The two most affected locations for pain and physical disability in adults are hip and knee [4]. Owing to its important prevalence worldwide, OA represents a huge burden for each affected individual, for public health resources utilization [5]. Based on their benefit:risk ratio, the use of chondroitin sulfate, diacereine, glucosamine sulfate, avocado/soybean unsaponifiables and hyaluronic acid has recently been considered as of potential interest for the symptomatic management of OA [6].

Structural modification of the joint is considered to be the most important determinant of OA progression. Several drugs and nutriceuticals have been evaluated as possible disease-modifying OA drugs, and their effects on progression of OA have been published in the past years. These include orally administered glucosamine sulfate, chondroitin sulfate, doxycycline, risedronate and diacereine, and intermittent courses of intra-articular injections of hyluronan [7–9].

Calcitonin (CT) is a polypeptidic hormone that has been recognized for more than 30 years as an inhibitor of osteoclast activity. Although the physiological role of calcitonin in humans has not been fully elucidated, many studies have suggested that it has beneficial effects on the clinical and biological disturbances of diseases that are characterized by excess bone remodeling [10].

For many years, it has been necessary to administer calcitonin parenterally by either intramuscular or subcutaneous injections. Unfortunately, there are several drawbacks to injection – it is nonphysiological and has no relationship to either the site or the rhythm of release of endogenous calcitonin. Furthermore, the effects of injectable calcitonin are poorly reproducible and not well accepted by patients. In some cases, the injection of calcitonin produces unpleasant reactions, including nausea, vomiting, flushing of extremities and vertigo.

The drawbacks of injectable calcitonin have stimulated interest in alternative routes of delivery. Substantial evidence of bioavailability and bioefficacy sufficient to produce clinical effects equivalent to those of parenteral administration of calcitonin are currently available for three alternative routes: nasal spray, oral administration and rectal suppository [11–13].

It was recently suggested that calcitonin might be an asset in the management of OA [14].

Oral administration of salmon calcitonin

A Phase I clinical trial was originally designed to verify that salmon CT (sCT) was absorbed to a significant extent and produced the biological and metabolic changes expected in healthy humans, in order to evaluate its absolute bioavailability and to confirm its short-term safety and tolerability. In this perspective, three single oral doses of sCT (0.4, 0.8 and 1.2 mg) were compared with an oral placebo and an intravenous infusion of 0.01 mg (50 IU) of sCT. The oral formulation consisted

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of tablets containing 0.4 mg of sCT and 225 mg of a caprylic acid derivative as a specific carrier, colyophilized from a sodium phosphate-buffered solution. The authors concluded that sCT was reliably absorbed from the oral formulation, with an absolute bioavailability of 0.5–1.4%, depending on the dose. It induced a marked, dose-dependent drop in blood and urine C-terminal telopeptide of type I collagen (CTX-I), with the effects of 1.2 mg exceeding those of 0.1 mg intravenously [11]. This oral formulation of sCT, based on the Eligen® technology (Emisphere Technologies, NJ, USA), which is comprised of the administration of an oral delivery agent in combination with an active macromolecule, was further investigated in a cohort of 277 healthy postmenopausal women. They received treatment with either daily 0.15, 0.4, 1.0 or 2.5 mg or intermittent doses (1.0 mg every other day) of sCT combined with the delivery agent (8-[*N*-2-hydroxy-5-chloro-benzoyl]-amino-caprylic acid, 200 mg) or placebo for 3 months. After the first dose, sCT evoked a dose-dependent decrease in serum CTX-I (from -60.8 to -81.8% from baseline) compared with placebo. The area under the curve of serum CTX-I responses at 1 and 3 months showed strong correlation with those at baseline. At month 3, the placebo-corrected changes in the predose value of serum and urinary CTX-I were significant only in the 1.0-mg dose group (-18.9 and -20.5%, respectively). The oral formulation was well tolerated, with mild-to-moderate gastrointestinal and skin manifestations, apparent mainly in the high-dose group. The authors concluded that this 3-month trial demonstrated that the novel Eligen technology-based oral formulation of sCT had a potential to become a safe and effective alternative to injectable CT in diseases where this compound could be of interest [15].

Calcitonin & preclinical models of osteoarthritis

The effects of different pharmacological concentrations (0, 5, 10, 100 and 1000 ng/ml) of synthetic human CT (hCT) and sCT, on the incorporation of [^{3}H]thymidine and production of proteoglycans (PG) and type II collagen (coll II) by human articular chondrocytes were studied in the 3D chondrocytes culture model. Incubation with hCT or sCT did not affect [^{3}H]thymidine uptake, regardless of the dose. PG and coll II release into culture medium, cluster content and total production increased significantly in a dose-dependent manner. Cumulative curves for these parameters showed a progressive significant increase with culture duration at hCT and sCT dose of 0, 5 and 10 ng/ml. Cumulative curves obtained with 10, 100 and 1000 ng/ml were seldom significantly different from one another. No differences emerged between the use of hCT or sCT. The authors concluded that sCT exerted no proliferative effect on human articular chondrocytes, but displayed a dose-dependent and prolonged stimulatory effect on PG and coll II production [16]. These results, obtained with supra-physiological doses of sCT and on a small number of samples, might be of marginal importance.

Salmon calcitonin, at concentration from 0 to 50 ng/ml, was also investigated in human osteoarthritic chondrocytes isolated from hips and knees. The spontaneous collagenolytic activity, measured using a radiolabeled coll II, was inhibited by sCT in a dose-dependent manner. Stromelysin and plasmin activity were unaffected by sCT, whereas chondrocyte phospholipase A2 activity was decreased by sCT. Chondrocyte preincubation with sCT significantly decreased the cell binding of labelled TNF-α, but did not affect IL-1 β-cell binding. Attachment of chondrocytes on fibronectin was markedly stimulated by sCT, while attachment to coll II was not. Significant effects were obtained using at least 2 or 5 ng/ml of sCT. The conclusion was that sCT appears to decrease collagenolytic activity by decreasing its activation and/or increasing its inhibition by tissue inhibitors of metalloproteinases. The authors suggested that sCT might act on osteoarthritic chondrocyte activation via mechanisms such as phospholipase A2 activity, human TNF-α or fibronectin receptor expression [17].

The preliminary findings of direct CT effects on isolated chondrocytes were supported in experiments on *ex vivo* cultures of articular explants, in which CT was demonstrated to attenuate the oncostatin M (OSM) and TNF-α-induced cartilage degradation [18]. The underlying mechanisms appear to involve attenuation of matrix metalloproteinase (MMP) expression and activity in articular chondrocytes, which appears to corroborate the findings by Hellio *et al.* [17–19]. In these studies, the CT receptor was identified in articular chondrocyte by immunochemistry and reverse transcriptase PCR. Calcitonin concentration dependently increased cAMP levels and isolated chondrocytes. Explants cultured with TNF-α and OSM showed a 100-fold increase in C-terminal telopeptide of coll II (CTX-II) release compared with vehicle-treated controls. The degradation of coll II in these explants was

concentration-dependently inhibited by CT, (65% protection at 10 nM CT). TNF-α and OSM induced a pronounced increase in MMP activity, which was strongly inhibited by CT [18]. It should be acknowledged that the concentrations of CT used by many investigators to show actions of CT on chondrocytes are relatively huge compared with those that are effective in an accepted CT target cell type, the osteoclast. This opens the possibility that CT is actually acting nonphysiologically on another receptor on chondrocytes. This possibility is increased by the suggestion that human cartilage and chondrocytes do not express the CT receptor [20].

Calcitonin & animal models of osteoarthritis

A well-established model of induced OA is the anterior cruciate ligament transection (ACLT) in dogs or rats, which is driven by instability of the knee leading to OA lesion that mimics consequences of traumatic injury in humans [18]. After ACLT, 12 dogs received a daily nasal spray delivering either 400 IU of sCT or a placebo. At day 84 after surgery, in the sCT treated group, OA lesions observed in the medial knee compartment of placebo-treated animals were significantly reduced. Bone mineral density and bone volume fraction assessed in different regions of interest of the subchondral cancellous bone of tibial plateau were decreased in placebo-treated animals, whereas they remain unchanged after CT treatment [21].

The same group, using a similar methodology, further investigated the effect of sCT on the response of bone, cartilage and synovium in the early stages of OA and the impact of sCT of the severity of the cartilage lesions. The sCT-treated dogs received a daily subcutaneous injection of sCT at a dose of 3 units per kg of bodyweight. Treatment was started on day 14 after surgery and was stopped either on day 48 or on day 104 after surgery. All ACLT joints developed OA. In contrast to sham-operated animals, all operated dogs exhibited an early and sustained rise in the levels of urinary pyridinium crosslinks and serum levels of keratan sulfate and hyaluronic acid. Calcitonin markedly reduced the levels of these markers and the severity of OA lesions. The length of time during which bone resorption was suppressed by CT was inversely related to the extent of cartilage macroscopic and microscopic damage, suggesting that subchondral trabecular loss itself may contribute to articular cartilage breakdown. The authors also speculate that, by reducing the rate of resorption of the bone subchondral support, CT might also decrease the mechanical stresses upon the overlying cartilage, as well as the 'leakage' of matrix molecules from the articular tissue. In their opinion, this might explain, at least in part, why this hormone reduces not only the serum levels of markers of synovial and cartilage metabolism, but also the enlargement of osteophytes [22]. These results were further confirmed with sCT administered as a nasal spray, delivering a daily dose of 400 IU. sCT also enhanced the hyaluronic acid content, as well as the size distribution and relative abundance of fast-sedimenting aggrecan aggregates in cartilage from both operated and nonoperated knees. In the sCT-treated group (dogs with ACLT), the cartilage content of keratan sulfate increased in operated joints, but not in nonoperated joints [23]. The relevance of this study may be marginal, due to the difference in bioavailability of nasal spray sCT compared with oral sCT.

In rabbits undergoing section of the cranial cruciate ligament, sCT was administered intramuscularly at the dose of 7 IU per day, following two regimens (i.e., daily from day 1 to week 8 postoperatively or daily from week 8 to week 16 postoperatively). sCT appeared to inhibit the progression of OA, in prophylactic stages. By increasing the layers of hyaline cartilage, restoring the cellular metabolism and decreasing the volume of osteophytes. In therapeutic stages, sCT had a healing effect by decreasing the subchondral cysts, regenerating the hyaline cartilage and restoring cellular metabolism [24]. In the nontraumatic model of OA induced by ovariectomy in rats, oral sCT (2 mg/kg) administered for 9 weeks resulted in a significant decline in the CTX-II released compared with vehicle-treated animals [18]. In the same model, the same group of investigators further demonstrated that the effect of CT on serum levels of CTX-II was similar to the one obtained by 17-β-estradiol supplementation. In the same investigation, histologic scoring of cartilage erosion showed significantly less cartilage erosion in CT-treated ovariectomized rats versus control ovariectomized rats that were untreated or treated with the carrier alone [25].

Calcitonin in clinical studies of osteoarthritis

To date, few studies have investigated the effect of CT in clinical trials involving patients with OA.

In a study involving 30 patients suffering from gonarthritis, nasal spray of sCT (2 × 100 IU/day), alone or combined with flavonoids, with or without naproxen sodium (2 × 550 mg/day) resulted

in an improvement in pain visual analog score and paracetamol consumption, and the effect lasted until 3 months after the withdrawal of sCT and/or naproxen [26].

The suppression of CTX-II, described in animals, was also observed in postmenopausal women aged 55–85 years. These subjects received treatment with different doses of oral sCT (0.15, 0.4, 1.0 or 2.5 mg daily) combined with the previously described Eligen technology-based carrier molecule (200 mg) or placebo for 3 months. Women who received 1.0 mg of sCT and were in the highest cartilage turnover tertile at baseline presented the greatest decrease in urinary CTX-II after 3 months of treatment. The effect of oral sCT in the lowest and middle tertiles of baseline urinary CTX-II was nonstatistically significant. The authors concluded that women with accelerated cartilage degradation at baseline (high CTX-II) appeared to be more responsive to clinically effective doses of oral sCT. Whereas it is well accepted that women with elevated baseline urinary CTX-II were most likely to later present with joint-related symptoms or manifest OA, it should be acknowledged that, globally, this study was a failure as no significant reduction in CTX-II was observed in the overall population. At any rate, this study was a *post hoc* analysis of a clinical trial investigating the efficacy and safety of sCT for the inhibition of bone turnover in postmenopausal women and, subsequently, the inclusion criteria were not in accordance with those usually requested for clinical trials conducted in OA [27].

Patients with OA of the medial tibiofemoral compartment, with a score of 3 on the Kellgren-Lawrence score and exhibiting enhanced uptake of the bone-seeking agent in the medial knee compartment on the delayed bone scan image after bone scintigram, were randomized to either placebo or 0.5 or 1 mg daily of oral sCT for 84 days in a double-blind trial. On day 84, patients in both the placebo group and the group receiving 1 mg of sCT exhibited a similar significant decrease in pain scores. However, a significant reduction in the function score was only observed in the two sCT groups. Significant reductions in the levels of stromelysin (MMP-3) and hyaluronan were observed in the two sCT groups. The group of patients receiving 1 mg of sCT also exhibited significant decreases in the levels of CTX-II, coll II neoepitope C2C and MMP-13. Notwithstanding the failure on the primary end point (pain score), the authors concluded that by improving functional disability and by reducing levels of biomarkers that are thought to be predictive of joint space narrowing, oral sCT at a dose of 1 mg might be a useful pharmacologic agent in human knee OA [28].

Conclusion

Calcitonin is a well-known polypeptidic hormone that has been investigated and used in the treatment of osteoporosis and other metabolic bone diseases for more than 30 years. In the mid-1980s, CT was considered as one of the main treatments of postmenopausal osteoporosis [10], until a pivotal study, which was expected to confirm its ability to reduce vertebral and nonvertebral fracture rates, led to at least equivocal conclusions [29]. These deceiving results, compared with the prohibitive costs of the nasal spray sCT, caused an almost total withdrawal of CT from the osteoporosis armomentarium. The demonstration that a new 'high-tech' oral formulation of sCT is absorbed and can mimic some of the effects of the parenteral administration of sCT resulted in an enthusiastic wave, supporting the idea that this particular formulation might no longer be useful in osteoporosis, whereas sCT appears rather weak compared with new therapeutic approaches, but may be useful in OA, where the field dramatically lacks unanimously and unequivocally recognised symptom- and structure-modifying agents. Whereas the *in vitro* and *in vivo* models of OA have provided some hints that sCT might exert some prophylactic or some beneficial effects on normal and osteoarthritic chondrocytes and/or a prophylactic and/or curative effect on artificially induced OA, the current evidence that oral sCT could be considered as an effective OA drug remains, at best, weak. In a *post hoc* analysis of a placebo-controlled study conducted in patients suffering from osteoporosis (and not from OA), oral sCT improved functional disability and markers of degradation. However, it failed to improve pain compared with placebo. CTX-II, which is thought to be a marker of cartilage degradation, was only reduced in women belonging to the highest tertile of cartilage turnover at baseline. Although these disappointing results do not preclude that, in a well-conducted, long-term, large-scale, randomized clinical trial, CT could potentially become an antiosteoarthritic drug, scientists and clinicians should remain extremely cautious, particularly when putting in perspective the encouraging results obtained, on surrogate markers, in osteoporosis and the disappointing results obtained from the main Phase III study on fracture rates. Another consideration that should not be forgotten when evaluating the global potential of the molecule links to the

cost-conscious use of health resources. Nasal CT was marketed at a daily price that precluded any positive cost–efficiency analysis, compared with any of the other marketed antiosteoporosis drugs. It is clear that, taking into account the potential wide study use of antiosteoarthritic medications, this aspect of the oral CT development also has to be taken into account.

Future perspective

For many years, the management of OA mainly concentrated on the symptomatic relief (pain and function). Over the past decade, much attention has been concentrated on the development of molecules that, besides their symptomatic activity, are also capable of stopping or slowing down the structural progression of the disease. With a better understanding of the pathophysiological processes underlying the development of OA and, owing to the emergence of new surrogates (i.e., MRI, biochemical markers of bone and cartilage turnover) to hard clinical end points (i.e., joint replacement), it is likely that a lot of resources will be invested in this area in the coming years. Oral CT could be one of the drugs that combines pain relief and inhibition of progression of OA, due to its well-documented analgesic effect and the positive indications coming from *in vitro* and *in vivo* studies. However, long-term, well-conducted, randomized, controlled trials are needed before investing too many expectancies in this product. In an experimental *in vivo* model of rheumatoid arthritis, CT synergized with prednisolone to elicit its anti-arthritic effects, which suggested that CT could be used as a novel cotreatment to augment efficacy and reduce side effects associated with the prolonged use of steroids [30].

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Executive summary

Mechanism of action

- Calcitonin is an established antiresorptive agent that has long been used for the treatment of osteoporosis.
- The effect of calcitonin on chondrocytes and cartilage metabolism is less investigated than in bone.
- The calcitonin receptor is expressed in articular chondrocyte at both the protein and mRNA level. The calcitonin receptor on the chondrocytes has been shown to be functional.

Pharmacokinetic & pharmacodynamic properties

- An Eligen® technology-based oral formulation of salmon calcitonin has been developed.
- The absolute bioavailability of this oral formulation is 0.5–1.4%, compared with intravenous infusion, depending on the dose.
- A dose of 1.0 mg daily of oral salmon calcitonin decreases serum and urinary levels of C-terminal telopeptide of type I collagen, confirming its ability to reduce bone turnover.
- Calcitonin stimulates proteoglycans and type II collagen synthesis by human chondrocytes.
- In bovine articular cartilage explants, calcitonin inhibits oncostatin M or TNF-α-induced matrix metalloproteinase expression.
- In various animal models, calcitonin inhibits the progression of the development of experimental osteoarthritis.

Clinical efficacy

- Oral salmon calcitonin reduces type II collagen telopeptide levels in postmenopausal women with high cartilage turnover at baseline.
- Compared with placebo, oral salmon calcitonin failed to reduce pain in women with active osteoarthritis of the knee. However, it provided a benefit in terms of functional capacity.

Safety & tolerability

- Outstanding safety of calcitonin, independent of the route of administration, has been documented for more than 30 years, hence a possibility of reassessment of its beneficial properties in human pathologies should be explored.

Bibliography

1 Goldring SR, Goldring MB: Clinical aspects, pathology and pathophysiology of osteoarthritis. *J. Musculoskelet. Neuronal Interact.* 6, 376–378 (2006).

2 Martel-Pelletier J, Lajeunesse D, Fahmi H, Tardif G, Pelletier JP: New thoughts on the pathophysiology of osteoarthritis: one more step toward new therapeutic targets. *Curr. Rheumatol. Rep.* 8, 30–36 (2006).

3 Garstang SV, Stitik TP: Osteoarthritis: epidemiology, risk factors, and pathophysiology. *Am. J. Phys. Med. Rehabil.* 85, S2–S11 (2006).

4 Arden N, Nevitt MC: Osteoarthritis: epidemiology. *Best Pract. Res. Clin. Rheumatol.* 20, 3–25 (2006).

5 Jinks C, Jordan K, Croft P: Osteoarthritis as a public health problem: the impact of developing knee pain on physical function in adults living in the community: (KNEST 3). *Rheumatology (Oxford)* 46, 877–881 (2007).

6 Bruyere O, Burlet N, Delmas PD, Rizzoli R, Cooper C, Reginster JY: Evaluation of symptomatic slow-acting drugs in osteoarthritis using the GRADE system. *BMC Musculoskelet. Disord*. 9, 165–174 (2008).

7 Reginster JY: The efficacy of glucosamine sulfate in osteoarthritis: financial and nonfinancial conflict of interest. *Arthritis Rheum*. 56, 2105–2110 (2007).

8 Bruyere O, Reginster JY: Glucosamine and chondroitin sulfate as therapeutic agents for knee and hip osteoarthritis. *Drugs Aging* 24, 573–580 (2007).

9 Brandt KD, Mazzuca SA: Lessons learned from nine clinical trials of disease-modifying osteoarthritis drugs. *Arthritis Rheum*. 52, 3349–3359 (2005).

10 Reginster JY: Calcitonin for prevention and treatment of osteoporosis. *Am. J. Med*. 95, S44–S47 (1993).

11 Buclin T, Cosma Rochat M, Burckhardt P, Azria M, Attinger M: Bioavailability and biological efficacy of a new oral formulation of salmon calcitonin in healthy volunteers. *J. Bone Miner. Res.* 17, 1478–1485 (2002).

12 Reginster JY, Jupsin I, Deroisy R, Biquet I, Franchimont N, Franchimont P: Prevention of postmenopausal bone loss by rectal calcitonin. *Calcif. Tissue Int*. 56, 539–542 (1995).

13 Reginster JY: Calcitonin: newer routes of delivery. *Osteoporos. Int*. 3, S3–S6 (1993).

14 Manicourt DH, Devogelaer JP, Azria M, Silverman S: Rational for the potential use of calcitonin in osteoarthritis. *J. Musculoskelet. Neuronal Interact*. 5, 285–293 (2005).

15 Tanko LB, Bagger YZ, Alexandersen P *et al.*: Safety and efficacy of a novel salmon calcitonin (sCT) technology-based oral formulation in healthy postmenopausal women: acute and 3-month effects on biomarkers of bone turnover. *J. Bone Miner. Res.* 19, 1531–1538 (2004).

16 Franchimont P, Bassleer C, Henrotin Y, Gysen P, Bassleer R: Effects of human and salmon calcitonin on human articular chondrocytes cultivated in clusters. *J. Clin. Endocrinol. Metab*. 69, 259–266 (1989).

17 Hellio MP, Peschard MJ, Cohen C, Richard M, Vignon E: Calcitonin inhibits phospholipase A2 and collagenase activity of human osteoarthritic chondrocytes. *Osteoarthr. Cartil.* 5, 121–128 (1997).

18 Sondergaard BC, Wulf H, Henriksen K *et al.*: Calcitonin directly attenuates collagen type II degradation by inhibition of matrix metalloproteinase expression and activity in articular chondrocytes. *Osteoarthr. Cartil.* 14, 759–768 (2006).

19 Karsdal MA, Tanko LB, Riis BJ *et al.*: Calcitonin is involved in cartilage homeostasis: is calcitonin a treatment for OA? *Osteoarthr. Cartil.* 14, 617–624 (2006).

20 Lin Z, Pavlos NJ, Cake MA *et al.*: Evidence that human cartilage and chondrocytes do not express caclitonin receptor. *Osteoporos. Int*. 16, 450–457 (2008).

21 Behets C, Williams JM, Chappard D, Devogelaer JP, Manicourrt DH: Effects of calcitonin on subchondral trabecular bone changes and on osteoarthritis cartilage lesions after acute anterior cruciate ligament deficiency. *J. Bone Miner. Res*. 19, 1821–1826 (2004).

22 Manicourt DH, Altman RD, Williams JM *et al.*: Treatment with calcitonin suppresses the responses of bone, cartilage, and synovium in the early stages of canine experimental osteoarthritis and significantly reduces the severity of the cartilage lesions. *Arthritis Rheum*. 42, 1159–1167 (1999).

23 El Hajjaji H, Williams JM, Devogelaer JP, Lenz ME, Thonar EJ, Manicourt DH: Treatment with calcitonin prevents the net loss of collagen, hyaluronan and proteoglycan aggregates from cartilage in the early stages of canine experimental osteoarthritis. *Osteoarthr. Cartil.* 12, 904–911 (2004).

24 Papaioannou NA, Triantafillopoulos IK, Khaldi L, Krallis N, Galanos A, Lyritis GP: Effect of calcitonin in early and late stages of experimentally induced osteoarthritis. A histomorphometric study. *Osteoarthr. Cartil.* 15, 386–395 (2007).

25 Sondergaard BC, Oestergaard S, Christiansen C, Tanko LB, Karsdal MA: The effect of oral calcitonin on cartilage turnover and surface erosion in an ovariectomized rat model. *Arthritis Rheum*. 56, 2674–2678 (2007).

26 Badurski J, Jeziernicka E, Naruszewicz K, Racewicz A: Comparative analysis of three treatment regimens for treating gonarthritis with calcitonin, naproxen and flavonoids based on EULAR criteria and visual analogue scale (VAS). *Pol. Tyg. Lek*. 50, 37–40 (1995).

27 Bagger YZ, Tanko LB, Alexandersen P *et al.*: Oral salmon calcitonin induced suppression of urinary collagen type II degradation in postmenopausal women: a new potential treatment of osteoarthritis. *Bone* 37, 425–430 (2005).

28 Manicourt DH, Azria M, Mindeholm L, Thonar EJ, Devogelaer JP: Oral salmon calcitonin reduces Lequesne's algofunctional index scores and decreases urinary and serum levels of biomarkers of joint metabolism in knee osteoarthritis. *Arthritis Rheum*. 54, 3205–3211 (2006).

29 Chesnut CH 3rd, Silverman S, Andriano K *et al.*: A randomized trial of nasal spray salmon calcitonin in postmenopausal women with established osteoporosis: the Prevent Recurrence of Osteoporotic Fractures Study. PROOF Study Group. *Am. J. Med*. 109, 267–276 (2000).

30 Mancini L, Paul-Clark MJ, Rosignoli G *et al.*: Calcitonin and prednisolone display antagonistic actions on bone and have synergistic effects in experimental arthritis. *Am. J. Pathol*. 170, 1018–1027 (2007).