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Review
Calcitonin is involved in cartilage homeostasis: Is calcitonin a treatment for OA?

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Summary

Objective: Osteoarthritis (OA) is the most common form of degenerative joint diseases and a major cause of disability and impaired quality of life in the elderly. Recent observations suggest that calcitonin may act on both osteoclasts and chondrocytes. The present review was sought to summarize emerging observations from the molecular level to the preliminary clinical findings of possible chondroprotective effects of calcitonin.

Method: This review summarizes peer-reviewed articles found using pre-defined search criteria and published in the PubMed database before January 2006. In addition, abstracts from the OsteoArthritis Research Society International (OARSI) conferences in the time period 2000–2005 have been included in the search.

Results: Ample evidence for the effect of calcitonin on bone resorption was found. Support for direct effects of calcitonin on chondrocytes on matrix synthesis and inhibition of cartilage degradation have been published. In addition, clinical evidence for the effect of calcitonin on cartilage degradation is emerging.

Conclusion: Several independent lines of evidence suggest a direct chondroprotective effect of calcitonin in addition to the well-established effect on bone resorption. Given the currently limited availability of chondroprotective agents, much expectation regards the ongoing clinical assessment of calcitonin therapy for the prevention and treatment of OA.

Key words: Articular cartilage, Calcitonin, Calcitonin receptor, MMPs, CTX-II, Chondrocytes, Osteoarthritis, Treatment.

Introduction

Osteoarthritis (OA) is the most common form of arthritis[1]. The hallmark of the disease is progressive degeneration of articular cartilage. Currently management of OA is symptomatic and targets the alleviation of pain and joint function. There are presently no uniformly accepted treatments that are considered to alter the course of OA.

Experimental and clinical observations suggest that the structural integrity of articular cartilage is dependent on normal subchondral bone turnover, intact chondrocyte function and ordinary biomechanical stresses[2,3]. Because there is a strong inter-relationship between the subchondral bone and the articular cartilage, an ideal therapeutic agent, in the face of normal biomechanical stresses, might logically be directed at regulating the metabolic activity of both bone and cartilage.

The key components of articular cartilage are type II collagen and aggrecan, which together constitute 90% of the dry weight of healthy cartilage[4]. Therefore, drug development strategies have focused on inhibition of the enzymes responsible for the degradation of these extra cellular matrix (ECM) molecules, such as the matrix metalloproteinases (MMPs)[5]. This paper reviews the potential physiological and pharmaceutical role of calcitonin in modifying the cartilage structure. This role of calcitonin in cartilage pathologies has only recently been introduced in the scientific literature, but since then a great amount of data has been published which justifies the importance of a new review to supplement what has been published earlier[6,7].

Methods

DATA SEARCH

The present review summarizes peer-reviewed publications found on PubMed without time limitations, searched until January 2006. In addition, abstracts from the OsteoArthritis Research Society International (OARSI) conferences in the time period 2000–2005 have been included in the search. The search topics were calcitonin, clinical trial, osteoclast, chondrocyte, articular cartilage, review, cartilage and pain. All combinations were used.

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Results

CALCITONIN AND THE CALCITONIN RECEPTOR

Calcitonin is an established anti-resorptive agent that has long been used for the treatment of osteoporosis[8]. Endogenously, calcitonin is produced by the parafollicular cells (C-cells)[9–11] in the thyroid gland. The calcitonin receptor (CTR) belongs to the B family of the G-coupled proteins receptors, also known as metabotropic receptors or seven transmembrane (7TM) receptors[12–15]. CTRs have been found in cells of many tissues and organs such as bone (osteoclasts), kidney, brain, lung, placenta, stomach, mammary gland, ovary, bone marrow and lymphocytes[12,14–17]. Studies have shown that binding of calcitonin to the CTR activates the adenylate cyclase/cAMP/protein kinase A (PKA) pathway and the Phospholipase C (PLC) pathway[14]. Additionally, studies have demonstrated activation of tyrosin phosphorylation, which serves to transduce downstream effectors such as mitogen activated protein kinase's (MAPK's) 44/42 activation[18] and activation of the Phospholipase D[12–14].

Most studies have focused on the function of the CTR in osteoclasts. The effect of calcitonin on chondrocytes and cartilage metabolism is less investigated.

IN VITRO EVIDENCE: CTR EXPRESSION AND SIGNALING

Recently it was demonstrated, that the CTR was expressed in articular chondrocytes at both the protein and mRNA level[19]. Further corroborating these interesting findings, exposure of chondrocytes to calcitonin, resulted in significant increased amounts of intracellular secondary messenger cAMP, which substantiated that chondrocytes have functional CTRs[19,20]. Although these findings support the concept of direct calcitonin-related effects on chondrocytes, the precise signal transduction pathways by which calcitonin exerts its effects need further investigation. In this context, investigations should target pathways involving members of the MAPK pathway, such as p38, p44/42, which previously have been proven important for calcitonin signaling in other cell-types[18].

IN VITRO EVIDENCE: CARTILAGE FORMATION

Serum levels of calcitonin are highest in newborn individuals and remain elevated during the first years of life[21,22]. High levels of calcitonin accompany the elongation of long bones, which grow via endochondral bone formation, and thus depend on proliferation, differentiation and maturation of chondrocytes. The peak of serum calcitonin coinciding high cartilage turnover is of particular interest when considering potential effects of calcitonin on mature cartilage metabolism. The notion is further nurtured by the findings of Khaldi *et al.* who recently demonstrated that salmon calcitonin (sCT) treatment of young growing rats – that have large amounts of growth plate chondrocytes – stimulated bone elongation[23].

Experimental evidence for a direct effect of calcitonin is predominantly obtained from cartilage experiments with chondrocytes or explants isolated from growth plate chondrocytes, distal femoral epiphyses rudiments, endochondral cartilage growth and hypertrophic chondrocytes[24–29]. Calcitonin had a direct effect on sulfate incorporation in lapine articular explants that was greater in OA cartilage than normal[30]. These experiments[24,25,29] demonstrated that calcitonin stimulates cartilage formation and maturation, further corroborating the effects of calcitonin during endochondral bone growth.

However, there are large differences between features of hypertrophic growth plate chondrocytes and articular chondrocytes. Nevertheless, studies by Franchimont *et al.* demonstrated that calcitonin dose-dependently stimulates proteoglycan synthesis and cellular proliferation *in vitro*, in isolated articular chondrocytes[31].

Even though these experiments eluted to a possible chondroanabolic action of calcitonin, a direct effect on intact articular chondrocytes has just recently been demonstrated in *ex vivo* cultures of articular cartilage explants[19]. In this more *in vivo* like biological model, calcitonin was shown to stimulate proteoglycan and collagen type II synthesis as estimated by incorporation of radioactive ^{35}S and ^{3}H proline, respectively. These findings are in alignment with the interesting findings of Malemud *et al.*[32], who demonstrated that small molecule modulators of cAMP, Forskolin or phosphodiesterase (PDE) inhibitors (IBMX), resulted in increased cAMP levels in chondrocytes, which was accompanied by an increase in proteoglycan synthesis, i.e. marked induction of aggrecan mRNA transcription. Thus, it seems reasonable to anticipate that calcitonin stimulates cAMP generation in chondrocytes, which in turn may result in increased synthesis of matrix molecules.

Taken together, these data suggest that calcitonin exerts important stimulatory effects during endochondral bone formation as well as for the maintenance of the homeostasis of mature articular chondrocytes.

IN VITRO EVIDENCE: INHIBITION OF CARTILAGE DEGRADATION

The effect of calcitonin on the catabolic activity of chondrocytes has received modest attention. The plausibility of direct actions of calcitonin on cartilage is supported by preliminary studies on isolated chondrocytes. In this experimental setting, calcitonin was shown to attenuate non-identified collagenase activity targeting collagen type II[33]. The authors speculated that this attenuation might be the result of countered tumor necrosis factor-α (TNF-α) signaling, yet the biochemical rationale for this has not been clarified.

Articular cartilage explants cultured in the presence of the catalytic cytokines, Oncostatin M (OSM) and TNF-α, were shown to be a useful *ex vivo* model of cartilage degradation[27,34] and allow assessment of direct effects of candidate drugs on cartilage degradation. Compared to cultures of isolated chondrocytes, this *ex vivo* model offers the advantage that organic links between chondrocytes and the surrounding ECM are preserved. The implications of the structural integrity of the ECM for functionality are emphasized by reports showing that certain cellular responses can no longer be elicited when cells lose their organic links to the matrix[35–38]. Thereby, the *ex vivo* articular cartilage explants model offers high *in vivo* likeness, and is the only experimental system that allows investigation of direct effects of growth factors on cartilage degradation.

The preliminary finding of direct calcitonin effects on isolated chondrocytes has recently found support in experiments on *ex vivo* cultures of articular explants, in which calcitonin was shown to attenuate the OSM and TNF-α induced cartilage degradation[20]. The underlying mechanisms seem to involve attenuation of MMP expression and activity in articular chondrocytes, which seems to corroborate the findings by Hellio *et al.*[33].

The direct effects of calcitonin on osteoclasts resulting in inhibition of bone resorption are well-established[12,14]. Several studies have suggested that various species of

calcitonin have different activities toward the human CTR. Salmon calcitonin belongs to the most potent families of the calcitonins with avian and teleost origin, in contrast to human calcitonin from the primate and rodent family[12,14]. Whether similar discrepancies of potency will apply to chondrocytes seems reasonable, albeit deserves more attention.

CALCITONIN DEFICIENT AND CTR DEFICIENT MICE

Recently sCT deficient and CTR deficient mice were investigated for their bone phenotype. A highly unexpected phenotype of the calcitonin and calcitonin gene related peptide (CT/CGRP) deficient mouse[39] was the lack of an osteoporotic phenotype, which was otherwise expected due to the well-established anti-resorptive effect of calcitonin[40]. Although the CT/CGRP deficient mice showed an increased resorptive response to parathyroid hormone (PTH), the most apparent phenotype of these mice was increased bone formation, which could not be ascribed to an increase in osteoblast number. In support of this finding, CTR haplo-insufficient mice[41] also have increased bone formation rates without any apparent increases in bone resorption. Since we have no direct evidence for the existence of the CTR in osteoblasts[15,42,43] but in bone only in osteoclasts[16], these data indicate that the role of calcitonin might indeed not be restricted to regulation of osteoclast function[44]. This conclusion leaves room for other physiological interpretations of calcitonin.

IN VIVO EVIDENCE FROM TRAUMATIC AND NON-TRAUMATIC MODELS OF CARTILAGE DESTRUCTION

Both traumatic and non-traumatic models have been used to investigate the effect of calcitonin on the development of OA and other degenerative joint diseases. Surgically induced instability models of OA are often used due to their rapid and uniform onset of the disease. A well-established model is the anterior cruciate ligament transection (ACLT) in dogs or rats, which are driven by instability of the knee leading to OA lesion that mimics consequences of traumatic injury in humans[45]. The characteristic macroscopic findings include osteophyte formation and cartilage ulcerations, whereas microscopic findings include cartilage fibrillation, cellular cloning, and diffuse hyper cellularity.

Estrogen deficiency accompanying the menopause has been shown to be associated with an increased incidence and severity of OA[46]. On the contrary, estrogen and hormone replacement therapy (ERT/HRT) has been suggested to decrease the incidence of OA in postmenopausal women[47,48]. These trends are in line with the findings of changes in levels of biochemical markers of cartilage turnover. Levels of collagen type II degradation fragments (CTX-II) are two-fold higher after the menopause, whereas HRT is associated with suppression of CTX-II levels to premenopausal levels[49]. A recent study in cynomolgus monkeys demonstrated OA like pathological changes within articular joints of ovariectomized animals, which were prevented by estrogen treatment[50]. In addition, ovariectomy of mature rats have shown to result in the development of pathological changes after 9 weeks similar in nature to the very early changes observed in human OA, where mild erosion and loss of proteoglycans are among the earliest changes[51,52]. The histological appearance of the knee articular cartilage in the ovariectomized (OVX) group differs from the appearance of articular cartilage in models such as ACLT and meniscal tear[53,54], where more severe erosive changes often can be observed. The changes in the knee cartilage observed after ovariectomy are relatively mild and may represent features of earlier or less aggressive disease, which are difficult to address in many of the other models of OA. Thus, the OVX model is particularly suitable for the study of early-stage OA[55].

Ovariectomy induces increases in CTX-II levels, which are most pronounced after 4–6 weeks. These early increases show close correlations with the subsequent histological signs of articular cartilage degradation[55,56]. This is in accordance with the findings obtained in clinical investigations, where CTX-II levels and its relative changes were shown to be closely associated with future damage of articular cartilage of the knee joint assessed radiographically[57–59].

Collectively, the combined use of traumatic and non-traumatic models may bring the best possible predictions for the clinical setting.

Primary findings by Badurski *et al.*, made in rabbits with either corticosteroid administration, or meniscectomy and immobilization of the hind leg all pointed in the direction that calcitonin may counter the progression of articular cartilage loss[60,61].

In alignment, very recently, in the preferred traumatic OA model, the dog ACLT model[62–64], calcitonin was shown to counter the progression of joint lesions[62]. The authors implicated inhibition of subchondral bone turnover as a likely mechanism conveying this beneficial effect on cartilage[62], which by some investigators is believed to be the most important part of the progression of OA[2]. But more intriguingly a direct effect of calcitonin on cartilage formation was also suggested, as a consequence to the essential findings in the non-operated knees, that calcitonin resulted in significant increased proteoglycan content. These data are in alignment with the aforementioned studies in cultured isolated chondrocytes and *ex vivo* explants and indicate that calcitonin may stimulate collagen type II and proteoglycan synthesis[24,25,29,31], suggesting potential anabolic effects of the hormone on cartilage.

In the estrogen deficiency non-traumatic model of OA induced by ovariectomy[55], which more resembles the slow progression of OA in humans, the effect of calcitonin was assessed by measuring serum levels of collagen type II degradation, CTX-II. Calcitonin was able to suppress CTX-II release from articular cartilage. Previous studies demonstrate that such decreases in this biomarker strongly correlate to structural changes associated with the development of OA[55,56,65]. In contrast to the complete suppression of circulating CTX-II levels, CTX-I – a marker of osteoclast-mediated bone resorption – decreased by 50% only. These findings might reflect a parallel impact of calcitonin on subchondral bone resorption, as previously reported by Behets *et al.*[62] and chondrocytes, which could act synergistically in the prevention of OA.

A further interesting finding of *in vivo* administration of calcitonin is that calcitonin treatment of growing rats resulted in augmented longitudinal growth of the skeleton, which seen in the light of the anti-resorptive effect of calcitonin is very interesting[66] as other anti-resorptives would lead to a decrease rather than an increase in longitudinal growth, as osteoclast function is essential for growth[67]. These data, as previous discussed data, indicate that the role of calcitonin might indeed not be restricted to regulation of osteoclast function[44], thereby indicating that the effects of

calcitonin on chondrocytes may be an important part of the physiological role of calcitonin.

IN VIVO EVIDENCE: FRACTURE REPAIR INCLUDING ENDOCHONDRAL BONE FORMATION

Chondrocyte biology may be considered an integral part of fracture repair. Thus, whether calcitonin positively affects the biological repair process is interesting in terms of cartilage biology.

In most, but not all fracture studies, calcitonin was shown to positively affect the formation and radiological appearance of the callus. The conclusion of these studies was that calcitonin positively affects endochondral ossification during fracture healing, causing an increase in cartilaginous callus and a faster maturation. Interestingly, in cases of primary fracture healing not involving processes of endochondral ossification, calcitonin did not produce positive effects[68].

CLINICAL TRAILS

Although the chondroprotective effects of calcitonin in humans remain to be demonstrated, Bagger *et al.* recently reported significant inhibition of not only bone resorption but also cartilage degradation in elderly women treated with a recently introduced oral formulation of calcitonin for 3 months[7].

Continuous treatment with calcitonin has been reported to result in loss of its inhibitory effect on bone resorption[69–72]. However, recent studies by Tanko *et al.* with an oral formulation of calcitonin in a clinical setting, showed similar potency at baseline and after 3 months of continuous treatment[73]. These differences remain to be further investigated.

Furthermore, an oral formulation of calcitonin was recently given to patients with knee OA in whom the clinical efficacy was assessed by Lequesne's algofunctional indices (ISK) and biochemical markers. Most convincingly, the investigators reported a decrease of more than 5 in the ISK score which is considered clinically relevant, a 40% decrease in serum MMP-13 accompanied with decreases in the urinary excretion of CTX-II[74]. These data are the first showing clinical efficacy of calcitonin in a diseased population monitored by several disease parameters, albeit responses in biochemical markers of cartilage degradation have been reported earlier[7].

ANALGESIC EFFECTS ON BONE PAIN

Calcitonin has unique analgesic effects on bone pain, which might relieve at least in part the symptoms accompanying joint diseases[75]. Calcitonin is effective in the treatment of osteoporosis[7,73,76] and in reducing the bone pain associated with osteoporosis and some bone tumors[75,77–79]. Pre-clinical evidence tends to support a direct, receptor-mediated action that is independent of opioid action[80,81]. Some evidence, however, has suggested a calcitonin interaction with opioid receptors[82–84]. In humans, similarities between calcitonin and morphine-induced analgesia, and reports of calcitonin-induced elevation of plasma β-endorphin levels, suggest the possible involvement of the endogenous opiate system in mediating the analgesic action of calcitonin[85]. The direct modulation of pain perception by calcitonin has been suggested to be through a central mechanism involving calcitonin-binding receptors in the central nervous system as well as an effect on local pain mediators through calcitonin-binding sites in the periphery[86]. Despite much details have been clarified, there is still no consensus regarding overall effects, albeit very recent systematical reviews on calcitonin on pain have substantiated the analgesic effects[87,88]. Further research is awaited to clarify whether calcitonin could improve the symptoms of joint disease and thereby the quality of life of patients.

OTHER MEDIATORS OF THE CTR STIMULATE CARTILAGE MATURATION

The CTR is promiscuous in terms of receptor binding and action, and activated albeit to a lesser extent by calcitonin gene related peptide (CGRP), amylin and adrenomedullin[14].

CGRP is expressed by neurons in close proximity of the growth plate[89,90], and although CGRP has been shown to lead to cAMP accumulation[91] the meaning of this finding is not completely clear. On the other hand the neuropeptides amylin and adrenomedullin, which have some similarities to calcitonin, have been shown to lead to longitudinal growth of the epiphyseal cartilage[92]. In addition, adrenomedullin has been shown to promote anabolic responses in both osteoblasts and chondrocytes[93].

Thus it appears that activation of the CTR either by calcitonin or by related peptides, has protective or anabolic effects on epiphyseal cartilage, and thereby promotes the longitudinal bone growth.

Discussion

Salmon calcitonin has demonstrated several properties that suggest it may be a clinically important therapeutic agent for OA. The analgesic properties may give fast symptom alleviation. From a structure modifying perspective, calcitonin has regulatory actions on cartilage and subchondral bone, which may cause long-term clinical benefits.

Progression of OA involves both articular cartilage changes, metabolic changes and changes in the remodeling of subchondral bone. In contrast to other anti-resorptive treatments calcitonin may restore both bone and cartilage turnovers. Many treatments have focused on the articular cartilage, while the most optimal treatment may be one that attacks both these metabolic imbalances.

As delineated in Fig. 1, evidences of both anabolic and anti-catabolic actions of calcitonin have been put forward. The CTR was recently identified on chondrocytes. In addition, in response to calcitonin, the classical 7TM receptor signaling though cAMP, in articular chondrocytes was demonstrated. The anti-catabolic effects of calcitonin have been shown to involve attenuation of MMP's expression and activity in articular chondrocytes[19,20]. Possible anabolic actions of calcitonin were shown to involve stimulation of proliferation, matrix synthesis and maturation of chondrocytes.

In conclusion, several lines of evidence, from *in vitro*, *ex vivo*, *in vivo* and preliminary clinical trails provide evidence that calcitonin treatment carries notable potentials for the prevention and treatment of degenerative joint diseases. Randomized clinical studies are needed to assess the herein hypothesized clinical benefits of calcitonin for the preservation of cartilage turnover and health.

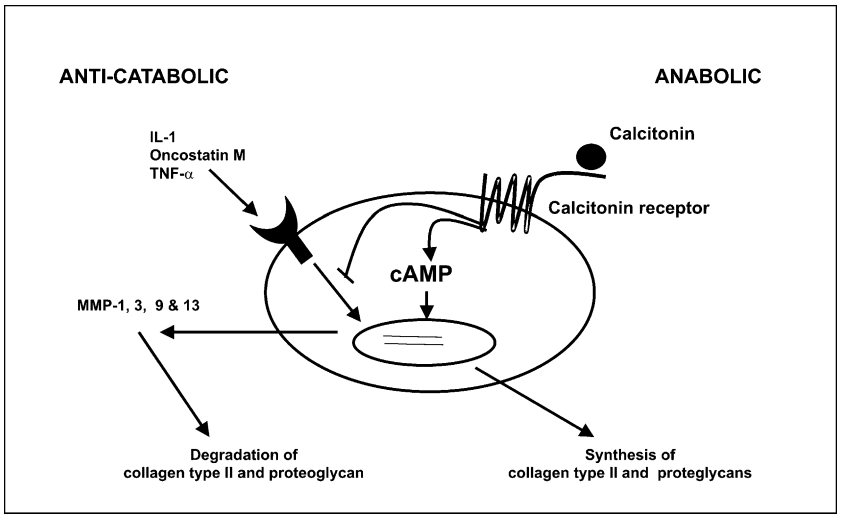

Fig. 1. Several catabolic cytokines such as interleukin-1 (IL-1), OSM and TNF-α have been shown to be important mediators of catabolic processes in chondrocytes. This results in the expression of a battery of catabolic MMPs, that in turn results in vast cartilage degradation. Recently the CTR was identified on articular chondrocytes in conjunction with cAMP signaling, verifying the presence of functional receptors. Anti-catabolic effects of calcitonin involve down-regulation of MMP activity which results in attenuated proteoglycan and collagen type II degradation. Anabolic effects of calcitonin have been identified as induction of collagen type II and proteoglycan synthesis. In combination, these important effects on articular chondrocyte metabolism may prove beneficial for cartilage health.

Author's disclosure

Karsdal MA, Riis BJ, Sondergard BC, Henriksen K, Qvist P and Christiansen C are employed at Nordic Bioscience. Karsdal MA, Riis BJ, Qvist P and Christiansen C are stockholders in Nordic Bioscience.

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