Osteoarthritis and Cartilage (1997) **5**, 121–128

1063–4584/97/020121 + 08 $12.00/0

OSTEOARTHRITIS and CARTILAGE

Calcitonin inhibits phospholipase A2 and collagenase activity of human osteoarthritic chondrocytes

BY M. P. HELLIO*, M. J. PESCHARD†, C. COHEN‡, M. RICHARD† AND E. VIGNON*

**Claude Bernard University, Pavillon F, Hôpital Edouard Herriot, 69437 Lyon cedex 03, France; †Department of Biochemistry, INSERM-CNRS U189, Lyon Sud Medical School, BP 12, 69921 Oullins Cedex, France; and ‡Laboratoires Specia, 16 rue Clisson, 75636 Paris, France*

Summary

Calcitonin (CT) is a known potent inhibitor of bone resorption but its effect on cartilage enzymatic degradation has been incompletely studied. Salmon CT, at a concentration of 0, 0.1, 0.25, 0.5, 2.5 and 50 ng/ml, was added at 24 or 72 h to the culture medium of chondrocytes from human osteoarthritic hips and knees. The spontaneous collagenolytic activity, measured using a radiolabeled type II collagen, was inhibited by CT in a dose-dependent manner. However, CT had no effect on the total collagenolytic activity assayed after APMA activation. Stromelysin and plasmin activity, measured by degradation of casein and a synthetic substrate, were also unaffected by CT. Chondrocyte phospholipase A2 activity, assayed using a labeled specific substrate, was decreased by CT. Chondrocyte pre-incubation with CT significantly decreased the cell binding of labeled TNFα, but did not affect IL-1β cell binding. Attachment of chondrocytes on fibronectin was markedly stimulated by CT, while attachment to type II collagen was not. Significant effects were obtained using at least 2 or 5 ng/ml of CT. CT appears to decrease collagenolytic activity by decreasing its activation and/or increasing its inhibition by tissue inhibitors of metalloproteinases (TIMP). CT might act on osteoarthritic chondrocyte activation via mechanisms such as phospholipase A2 activity, human necrosis factor-α or fibronectin receptor expression.

Key words: Chondrocytes, Phospholipase A2, Degradative enzyme, Adhesion.

Introduction

CARTILAGE destruction is only one of the lesions of osteoarthritis (OA), but is regarded as the most important one for the progression of the disease. Biochemical alterations in the extracellular matrix, including proteoglycan loss and a loosening of the collagen network, are implicated in OA cartilage destruction. Alterations of collagen and proteoglycan in OA cartilage are likely mediated by an increased degradation, by collagenase and stromelysin, respectively [1]. The regulation of the synthesis and activity of these matrix metalloproteinases (MMPs) may occur at several levels. MMPs are secreted extracellularly as inactive proenzymes and activated in the matrix via an enzymatic cascade. Serine proteases, like the plasminogen activator/plasmin system, have been suggested to be the main activators of MMPs [2]. Once activated, the MMPs are inhibited by tissue inhibitor of metalloproteinases (TIMP), and the excessive degradation of matrix in OA is considered to result from an imbalance between the mechanisms of activation and inhibition of MMPs [3].

The mechanism of the dysregulation of MMPs in OA cartilage is still unknown and probably complex. Cytokines, such as interleukin-1 (IL-1) and tumor necrosis factor-α (TNF-α) are likely to be involved. Both these cytokines induce the synthesis of MMPs [4–6] and have been detected in the synovial fluid from OA patients [7]. OA chondrocytes were also shown to have a higher sensitivity to the stimulation of MMPs synthesis by IL-1 and TNF-α than normal cells [8, 9]. For both these cytokines, the high sensitivity was attributed to an increased level of both IL-1 and TNF-α receptors expressed in the OA chondrocytes. The cytokines also induce phospholipase A2 (PLA2) activity, an inflammatory enzyme increased in OA cartilage [10] and implicated in the regulation of collagenase activity [11].

Cell adhesion to extracellular matrix components, such as fibronectin and collagen, is another possible mechanism implicated in the regulation of protease synthesis. Many of the effects of the extracellular molecules on cell activity are exerted

Submitted 27 December 1995; accepted 2 July 1996
Supported by a grant from Rhone-Poulenc, Specia, FRANCE.
Address correspondence to: Pr E. VIGNON, Pavillon F, Hôpital Edouard Herriot, Place d'Arsonval, 69437 Lyon Cedex 03-France.

via the integrins receptors which are expressed by chondrocytes [12, 13]. Induction of MMPs synthesis by signal transduction through chondrocyte integrin receptors has been reported [14]. Elevated levels of fibronectin, demonstrated in OA cartilage [15] also suggest that cell–matrix interaction could be of importance in the regulation of OA cartilage cells.

Calcitonin (CT) is mainly known as a potent inhibitor of osteoclastic bone resorption, which implicates bone attachment of osteoclasts and enzymatic degradation. The degradative process associates synthesis of various proteases and MMPs, activation of inactive proenzymes and inhibition of active enzymes [16–22]. CT is known to induce osteoclast retraction [23] and to interfere at least with some steps of the enzymatic process of bone resorption [17–19].

There are some reported studies on the effects of CT on articular cartilage. *In vitro*, CT was found to stimulate proteoglycan and collagen synthesis in animal epiphyseal cartilage [24–27] as well as in rabbit and human articular cartilage [28, 29]. The study of CT in the treatment of experimental OA gave conflicting results. CT was found to prevent cartilage destruction in rabbits treated with steroids, partial menisectomy or joint immobilization [30], but no effect on cartilage was observed in another menisectomy experiment [31].

In the present study, cultures of human OA chondrocytes were used to determine whether CT could inhibit collagenase and stromelysin activity. The effect of CT on plasmin activity, PLA2 activity, cell binding of IL-1 and TNF-α, and cell adhesion to fibronectin and collagen, as regulatory mechanisms of MMPs activity was also investigated.

Materials and Methods

CHONDROCYTE CULTURE

Hips and knees of patients with OA were obtained at the time of joint replacement. Immediately after surgery, the joints were washed with a sterile cold physiologic saline solution and kept for 12 h at 4°C in a Gey saline solution (Gibco). The grossly fibrillated cartilage surrounding the area of bone exposure was dissected and cut into approximately 1 mm^3 fragments in the Gey solution.

Chondrocytes were released by sequential enzymatic digestion with 0.2% trypsin (Gibco) for 30 min, and then 6 h with 0.2% collagenase (Sigma, type IV) at 37°C in Gey solution. Cells were obtained after filtration with a Blutex 82 μ and centrifugation at 1100 *g* for 5 min [11]. They were counted using a Thoma cell. The cells were cultured at high density (8×10^6/ml) in a liquid suspension of HAM F12 medium (Gibco) containing 10% Ultroser (IBF) and antibiotics (penicillin and streptomycin 10 000 U/ml) in a 95% air–5% CO_2 atmosphere at 37°C. The culture medium was eliminated by centrifugation (1100 *g* for 5 min) after 24 or 72 h.

The cells were then seeded at high density (4×10^6 cells/0.5 ml) and cultured as described for 24 or 72 h in the presence of various concentrations (0, 0.1, 0.25, 0.5, 2, 5 and 50 ng/ml) of a synthetic salmon CT (CALSYN, Specia). Because gelatin was present in the furnished solution of the hormone, it was added at a similar concentration in the control medium. At the end of the culture period, the cells and the medium were separated by centrifugation (1100 *g* for 5 min). For enzymatic assays, cells were sonically disrupted (Sonimas type 25 T probe at 125 v) by three 15 s cycles in 50 mM Tris-HCL (pH 7.5). In repetitive control experiments, the cells did not divide and kept a rounded shape at the end of the culture period.

DETERMINATION OF CASEINASE ACTIVITY

Casein, a substrate of stromelysin, was used for the determination of stromelysin activity according to a previously reported method [32]. The spontaneously active caseinase activity was determined using an incubation mixture made of: 200 μl of cell homogenate or culture medium, 30 μl of buffer (0.416 M Tris HCl, pH 7.5, 1.25 M NaCl, 0.083 M $CaCl_2$, 0.166% NaN_3, 0.83% Triton X-100), 50 μl of 0.5% casein fluorescein isothiocyanate. Caseinolytic activity was also determined after activation, obtained by adding 10 μl of 0.17 M amino-phenyl-mercuric acetate (APMA). After incubation at 35°C for 4 and 24 h for the two sets of experiments, respectively, 280 μl of 5% trichloracetic were added. The precipitate was eliminated by centrifugation. A 0.50 M Tris-HCl pH 8.5 buffer (2 ml) was added to 250 μl supernatant. Fluorescence was determined using a fluorimeter at λ Ex = 365 nm and λ Em = 525 nm and expressed in units of fluorescence per hour of incubation and milligram of protein. Protein assay was done as previously described [33].

DETERMINATION OF COLLAGENASE ACTIVITY

Digestion of ^{3}H-labeled type II collagen (Bioethica, Lyon), was done as previously described [10, 32]. To determine the spontaneous collagenolytic activity, the incubation mixture consisted of: 200 μl cell homogenate or culture medium, 30 μl of

0.42 M Tris-HCl buffer; pH 7.5 [1.25 M NaCl, 0.083 M $CaCl_2$, 0.66% (w/v) sodium azide and 0.83% (w.v) Triton X-100, 20 µl of ^3H acetyl-collagen (about 100 000 dpm)]. To determine the total activity of the enzyme, 10 µl of 0.17 APMA were added to the mixture. After incubation at 35°C, for 24 h without APMA and 4 h with APMA, the reaction was stopped by adding 100 µl collagen (1.5 mg/ml), 0.04 M EDTA and 350 µl Dioxan. After centrifugation, the radioactivity of the supernatant was determined. Several blanks, made using the same incubation mixture without the enzyme extracts, were used to evaluate the spontaneous radioactivity obtained in the absence of enzymatic collagen degradation.

DETERMINATION OF PLASMIN ACTIVITY

Plasmin activity was determined using a kinetic method by measuring (at 405 nm) the cleavage of a synthetic substrate (D-Val-Leu-Lys p nitroanilide, Sigma) spectrophotometrically [34]. The incubation mixture consisted of: 200 µl cell homogenate or culture medium, 700 µl buffer (0.1 M Tris-HCL pH 7.5, 0.1% Tween-80, 0.001% NaN_3), 100 µl of 3 mM substrate. Results were expressed as Δ d.o. per minute of incubation and milligram of protein.

DETERMINATION OF PLA2 ACTIVITY

The assay was performed using a previously described method [10], using unlabeled L-α-phosphatidyl-ethanolamine and labeled 1-palmitoyl-2-($1^{14}C$) linoleyl-L-3-phosphatidyl-ethanolamine (NEN) as substrate. Briefly, 100 nmol of substrate (20 000 dpm), 3% sodium deoxycholate (50 µl), 0.25 M Tris-HCl buffer (200 µl) pH 7.5, 0.1 M $CaCl_2$ (50 µl) and 200 µl of culture medium or cell homogenate were incubated for 4 h at 37°C. [^{14}C]-linoleic acid was then extracted with Dole's reagent (isopropanol 40p., heptan 10p., H2SO4 N 1p.). Radioactivity was counted with 10 ml toluene based scintillation fluid. PLA2 activity was calculated by subtracting the value at 0 min and the nonenzymatic degradation of the substrate measured in blanks. Results were expressed as disintegrations per minute per hour of incubation and per milligram of protein.

CELL BINDING OF TNFα AND IL1β

Chondrocytes were cultured with increasing concentrations of calcitonin for 24 or 72 h, as described above. Cell suspensions (200 µl) were then incubated in HAM F12 with [^{125}I]-IL1β or TNFα (respective specific radioactivity 51.8 and 37 GBq/µM, NEN), at final concentrations of 310 and 250 pM for 4 h at 4°C, respectively [35]. Cells were then washed three times and centrifuged, and their radioactivity was measured using liquid scintillation.

CELL ADHESION

Chondrocytes were cultured with increasing concentrations of calcitonin for 72 h, as described above. Ninety-six-well microtitration plates were coated with fibronectin (20 µg/ml) or type II collagen (100 µg/ml) for 12 h at 4°C. Unoccupied binding sites were then blocked by incubating the plates for 2 h at 24°C with 1% bovine serum albumin (SIGMA, low endotoxin, globulin free). Cells (10^6/ml) were incubated in the coated wells for 1 h at 37°C. Unattached cells were eliminated by lavage. Attached cells were fixed with 1% glutaraldehyde (15 min, 24°C) and stained with 0.1% violet crystal for 30 min. The stained cells were treated with 0.2% Triton × 100 for 10 min and its intensity measured at 570 nm with a spectrophotometer [36].

STATISTICAL ANALYSIS

Cartilage from 48 joints (42 knees and six femoral heads) was used. Results obtained with knee or hip joints were similar. For each enzyme or binding assay, cells deriving from only one joint were used to evaluate the seven concentrations of CT (from 0–50 ng/ml) and samples from six different joints were tested. The effect of CT concentration was assayed in triplicate. Data were not selected. The differences between groups of increasing CT concentration were studied with an analysis of variance. When a significant difference was found, Student's paired t test was used to compared the calcitonin groups with the control group.

Results

EFFECT OF CT ON DEGRADATIVE ENZYMES

The spontaneous collagenolytic activity of chondrocytes was significantly ($P < 0.05$) decreased by CT both after 24 and 72 h of incubation (Table I). The minimum effective concentration was 2 ng/ml for 24 h and 5 ng/ml for 72 h [8.5 ($P = 0.03$) and 33.4% ($P = 0.002$) decrease, respectively]. The spontaneous collagenolytic activity of the medium was also inhibited by CT, the minimum effective concentration being 50 ng/ml for 24 h (32.7% decrease, $P < 0.001$) and 5 ng/ml for 72 h (16% decrease, $P < 0.01$). The collagenolytic

Table I

Effect of various concentrations of calcitonin on the cell and medium collagenolytic activity (Coll) of human osteoarthritic chondrocytes in culture

		Calcitonin (ng/ml)						
		0	0.1	0.25	0.5	2	5	50
Cell active Coll.								
24 h Incubation	Mean	6495	6585	6071	6028	5001*	4321*	3728*
	SD	1125	1007	474	231	478	452	534
72 h Incubation	Mean	7668	7766	7828	7486	7013	6991*	3898*
	SD	447	488	391	474	650	453	501
Medium active Coll.								
24 h Incubation	Mean	4488	4305	4266	4425	4520	4273	3020*
	SD	751	477	417	534	388	651	548
72 h Incubation	Mean	7870	7641	7571	7226	7120	5375*	4865*
	SD	866	594	616	422	416	503	499
Cell total Coll.								
24 h Incubation	Mean	15583	15346	15786	14983	14625	14656	13280
	SD	2370	2248	1911	2131	1595	1587	1965
72 h Incubation	Mean	15770	16060	15546	15378	14852	14821	12170
	SD	2582	2268	2078	2151	1933	2037	1688
Medium total Coll								
24 h Incubation	Mean	8960	8555	8655	8486	8343	8370	8106
	SD	674	588	480	522	474	367	605
72 h Incubation	Mean	12135	13080	12346	12598	13045	11745	9065*
	SD	1153	1398	1413	1607	1279	993	938

Collagenolytic activity is expressed in disintegrations per minute per hour of incubation and per milligram of protein. Active Coll. is the spontaneously assayed activity. Total Coll. is the activity assayed after APMA activation. *$P < 0.05$.

activity was increased by nearly 100% by APMA activation. The APMA-activated collagenolytic activity was unmodified by CT, with exception of a 25.2% decrease obtained at a concentration of 50 ng/ml after 72 h ($P < 0.001$).

The activity of both caseinase and plasmin was detected in both cells and medium, and increased with the time of culture. However, calcitonin had no clear effect on the activity of the enzymes (data not shown).

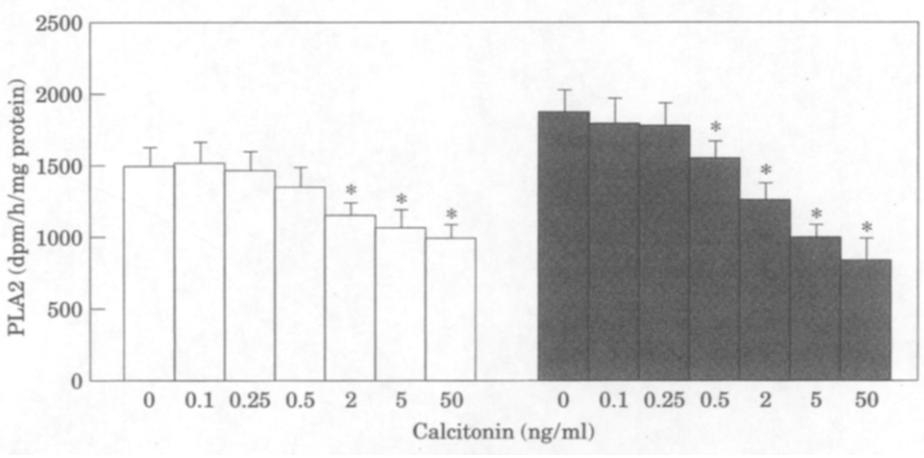

FIG. 1. Phospholipase A2 (PLA2) activity of cultured chondrocytes with increasing concentrations of salmon CT. Cells were incubated with CT for 24 (□) or 72 h (■). Enzyme activity is expressed as disintegrations per minute per hour of incubation and per milligram of protein. Mean + SD. *$P < 0.05$.

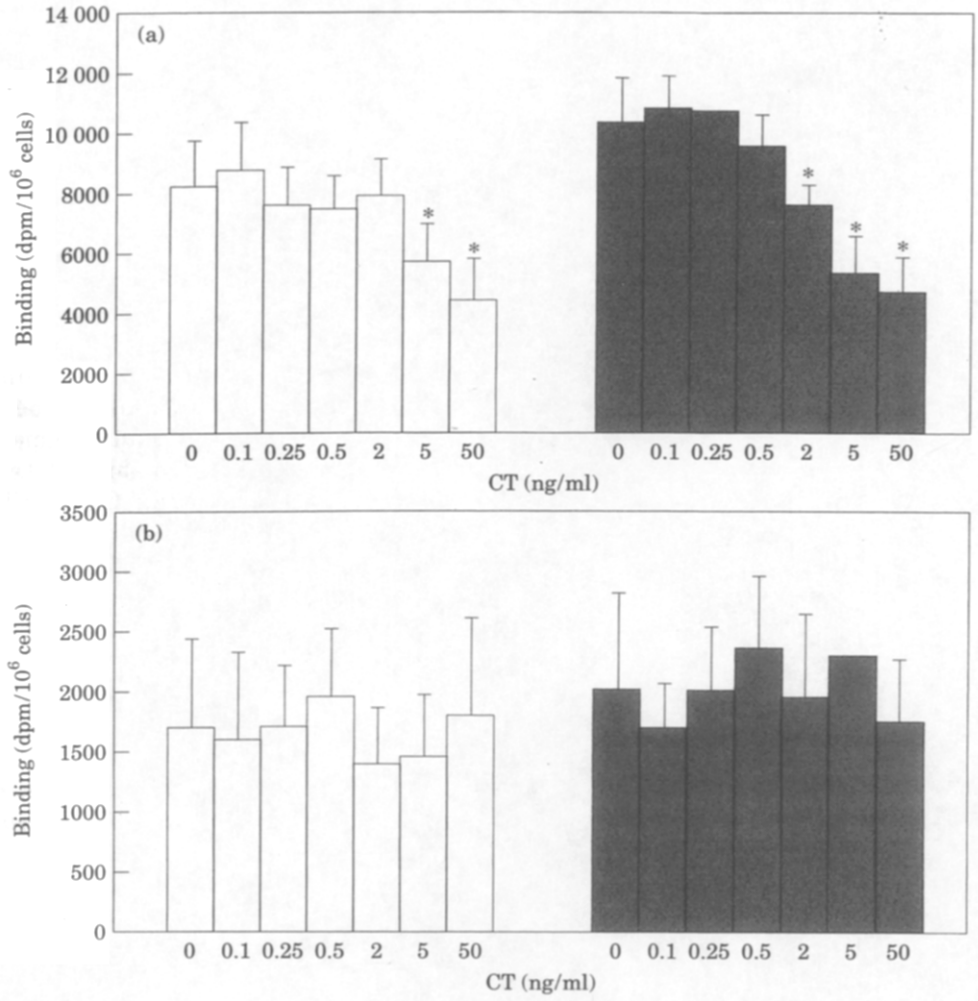

FIG. 2. Binding of (a) interleukin-1β and (b) tumor necrosis factor-α on cultured chondrocytes with increasing concentration of CT. Cells were preincubated for 24 (□) or 72 h (■) with CT before incubation with cytokines for 4 h at 4°C. Binding is expressed as disintegrations per minute per 10^6 cells. Mean + SD. *$P < 0.05$.

The effect of CT, incubated for 24 or 72 h, on cell PLA2 activity are represented in Fig. 1. Cell PLA2 was inhibited by CT, in a concentration dependent manner, both after 24 and 72 h of incubation. A statistically significant inhibition was obtained using a minimum of 2 ng/ml ($P < 0.02$) after 24 h and 0.5 ng/ml after 72 h ($P < 0.01$). The most significant decrease was 53.9% (50 ng/ml, 72 h). Soluble PLA2 was also significantly ($P = 0.04$) decreased with a minimum concentration of 2 ng/ml CT after 72 h of incubation. It remained unchanged after 24 h of incubation with CT.

EFFECT OF CT ON THE BINDING OF CYTOKINES

The results are shown in Fig. 2. The binding of radiolabeled cytokines was specific because it was found to be suppressed by cold cytokines. Cell preincubation with CT inhibited the binding of TNFα on chondrocytes. A significant decrease was obtained with a minimum of 5 ng/ml for 24 h (31.2%, $P < 0.01$) and 2 ng/ml for 72 h (26.9%, $P = 0.004$). The results contrast with the absence of effect of CT on the cell binding of IL-1β. In other experiments, no effect of CT on cytokine binding was found when both CT and cytokines were added at the same time in the culture medium.

EFFECT OF CT ON CELL ADHESION

The results are shown in Fig. 3. CT was found to enhance chondrocyte adhesion to fibronectin. A concentration of 5 ng/ml, which was the significant minimum effective value, increased cell adhesion by 35.4% ($P = 0.008$). The effect was selective because CT did not modify cell adhesion to Type II collagen in similar conditions. In other experiments, addition of CT at the time of cell incubation did not modify cell adhesion to fibronectin.

Discussion

CT is a known potent inhibitor of osteoclastic bone resorption. Its mechanism of action is not fully understood but osteoclast retraction and inhibition of degradative enzymes have been implicated [17–19, 22]. This study shows that the hormone may also interfere with the enzymatic degradation of OA cartilage by inhibiting collagenase activity.

The determined collagenolytic activity was likely attributable to collagenase. It has previously been shown to be similar to that obtained with a pure collagenase [37], and in the present study, markedly increased after APMA activation. Collagenolytic activity inhibition can be caused by a decrease in synthesis and/or activation of collagenase, or by an increased inhibition of collagenase by TIMPs. The synthesis of collagenase was probably unaffected by CT because the hormone did not modify the total collagenolytic activity obtained by APMA activation. The direct effect of CT on collagenase was not evaluated, and an effect of APMA on the hormone could be possible. However, this result is in agreement with a previous study showing that procollagenase synthesis was unmodified by CT in culture of bone tissue [14]. The possibility of an inhibition of procollagenase activation by CT is not supported by the lack of effect of the hormone on plasmin activity. In fact, plasmin is known to be a potent activator of collagenase [38], and has been suggested to be the main activator of MMPs in OA cartilage [2]. Interestingly, plasmin activity was also reported to be unaffected by CT in bone culture [16]. However, CT inhibition of procollagenase activation could be mediated by other lysosomal proteases because some of them were found to be inhibited by CT in bone tissue experiments [17, 18, 20]. CT inhibition of collagenolytic activity could also be related to an increased formation of TIMP, a finding previously reported in normal rabbit articular cartilage [39].

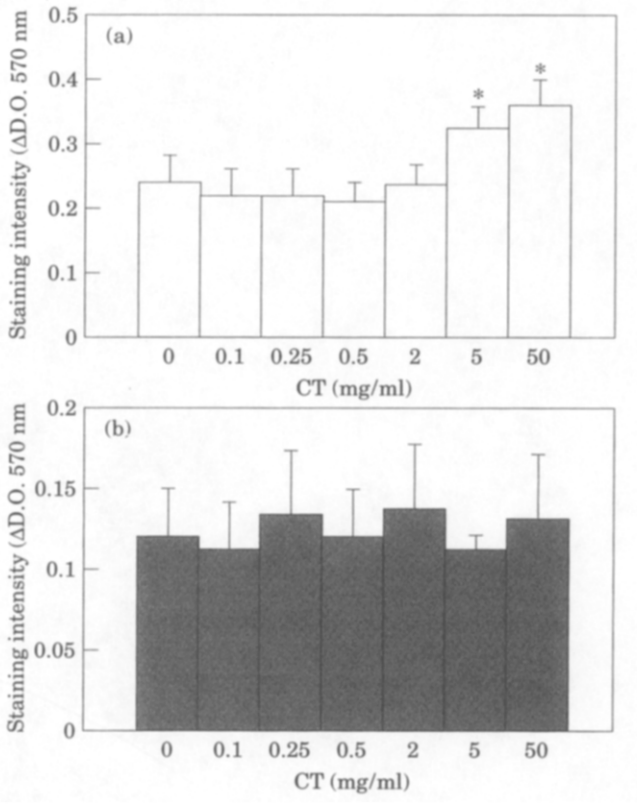

FIG. 3. Chondrocyte adhesion to (a) fibronectin and (b) type II collagen with increasing concentrations of calcitonin (CT). Cells were preincubated with CT for 72 h before incubation in wells coated with fibronectin and collagen for 1 h at 37°C. Adherent cells were stained with crystal violet and staining intensity (D.O.) was measured by spectrophotometry at 570 nm. Mean + SD.

Collagenase activity is known to be stimulated by cytokines such as IL-1 and TNFα. An increased stimulation of matrix degradation by IL-1 and TNFα was reported in OA cartilage and attributed to a higher level of receptors for both cytokines on OA chondrocytes [8, 9]. Inhibition of collagenase activity by CT could be related to an interaction of the hormone with the receptors of cytokines. Both radiolabeled IL-1 and TNFα were found to bind to cultured OA chondrocytes. CT did not modify the binding of IL-1 but significantly reduced the binding of TNFα. The effect was observed after the preincubation of cells with CT, but was not found when CT and TNFα were incubated together at the same time. This suggests that CT might decrease expression of TNFα receptors. This possible outcome was not investigated here.

A significant inhibition of PLA2 activity in OA cartilage was demonstrated in this study, and to our knowledge, is the first demonstration of this hormonal effect. Both the cellular and the soluble form of PLA2 were inhibited with a low concentration of CT. PLA2 activity was studied as a possible activator of collagenase activity, as previously demonstrated in culture of OA chondrocytes [11]. It is known to be up-regulated by IL-1 and TNFα stimulation [40], and was found to be increased in human OA cartilage [10]. A down-regulation of an increased PLA2 activity by CT could, therefore, be related to the observed decrease in collagenolytic activity, but confirmation of this mechanism would need further investigation. CT-induced inhibition of PLA2 might also explain the anti-inflammatory effects of the hormone, which were repeatedly reported and explained by various mechanisms including prostaglandin formation [41–45].

Cell adhesion to extracellular matrix components is another mechanism implicated in the regulation of cell activity. Chondrocyte attachment to a variety of matrix proteins, such as fibronectin and collagen, has been shown to be mediated by integrins [12, 13]. Fibronectin was found to inhibit collagen and proteoglycan synthesis in chondrocyte cultures [40], and signal transduction through chondrocyte integrin receptors was shown to up-regulate the expression of MMPs [14]. CT did not affect cell adhesion to collagen but significantly increased chondrocyte adhesion to fibronectin. This finding was unexpected, and apparently, cannot be related to the regulation of collagenase activity by CT.

Speculation on the clinical relevance of the present *in vitro* findings could be of interest. The biological activity of CT may vary with its source, and salmon CT has been suggested to be the most active hormone [41]. The maximum serum concentration of CT following s.c. administration of 100 UI of salmon CT approximated 0.4 ng/ml [42]. In the present work, and in other reported studies, a significant effect on chondrocyte culture was generally obtained with 5 ng/ml CT. PLA2 inhibition alone was detectable using 0.5 ng/ml CT. Thus, a high dosage of CT would probably be necessary *in vivo* to induce some effect on OA joints.

References

1. Dean DD. Proteinase-mediated cartilage degradation in osteoarthritis. Semin Arthritis Rheum 1991; 20 (suppl 2): 2–11.
2. Collier S, Ghosh P. The role of plasminogen in interleukin-1 mediated cartilage degradation. J Rheumatol 1988; 15: 1129–37.
3. Pelletier JP, Mineau F, Faure MP, Martel-Pelletier J. Imbalance between the mechanisms of activation and inhibition of metalloproteinases in the early lesions of experimental osteoarthritis. Arthritis Rheum 1990; 33: 1466–1476.
4. Pujol JP, Loyau G. Interleukin-1 and osteoarthritis. Life Sci 1987; 41: 1187–98.
5. MacNaul KL, Chartrain N, Lark M, Tocci MJ, Hutchinson NI. Discoordinate expression of stromelysin, collagenase and tissue inhibitor of metalloproteinases-1 in rheumatoid synovial fibroblast. Synergistic effects of interleukin-1 and tumor necrosis factor-α on stromelysin expression. J Biol Chem 1990; 265: 17238–45.
6. Malfait AM, Verbruggen G, Veys EM, Lambert J, DeRidder L, Cornelissen M. Comparative and combined effects of interleukin 6, interleukin 1β and tumor necrosis factor α on proteoglycan metabolism of human articular chondrocytes cultured in agarose. J Rheumatol 1994; 21: 314–20.
7. Vignon E, Balblanc JC, Mathieu P, Louisot P, Richard M. Metalloprotease activity, phospholipase A2 activity and cytokine concentration in osteoarthritis synovial fluids. Osteoarthritis 1993; 1: 115–20.
8. Martel Pelletier J, McCollum R, Dibattista J, Faure MP, Chin JA, Fournier S, Sarfati M, Pelletier JP. The interleukin-1 receptor in normal and osteoarthritic human articular chondrocytes. Identification as the type I receptor and analysis of binding kinetics and biologic function. Arthritis Rheum 1992; 35: 530–40.
9. Westacott CL, Atkins RM, Dieppe PA, Elson CJ. Tumor necrosis factor-α receptor expression on chondrocytes isolated from human articular cartilage. J Rheumatol 1994; 21: 1710–15.
10. Vignon E, Mathieu P, Louisot P, Vilamitjana J, Harmand MF, Richard M. Phospholipase A2 activity in human osteoarthritis cartilage. J Rheumatol 1989; (suppl 18), 16: 35–38.
11. Richard M, Vignon E, Peschard MJ, Broquet P, Carret JP, Louisot P. Serotonin-stimulated phospholipase A2 and collagenase activation in chondrocytes from human osteoarthritic articular cartilage. FEB Lett 1991; 278: 38–40.
12. Salter DM, Hugues DE, Simpson R, Gardner DL. Integrin expression by human articular chondrocytes. Br J Rheum 1992; 31: 231–234.
13. Loeser RF. Integrin-mediated attachment of articular chondrocytes to extracellular matrix proteins. Arthritis Rheum 1993; 36: 1103–1110.
14. Arner EC, Tortorella MD. Signal transduction through chondrocyte integrin receptors induces matrix metalloproteinase synthesis and synergizes with interleukin-1. Arthritis Rheum 1995; 38: 1304–1314.
15. Chevalier X. Fibronectin, cartilage and osteoarthritis. Semin Arthritis Rheum 1993; 22: 307–318.
16. Hamilton JA, Lingelbach S, Partridge NC, Martin TJ. Regulation of plasminogen activator production by bone resorbing hormones in normal and malignant osteoblasts. Endocrinology 1985; 116: 2186–2191.
17. Lerner UH, Jones IL, Gustafson GT. Bradykinin, a new potential mediator of inflammation-induced bone resorption. Studies of the effects on mouse calvarial bones and articular cartilage *in vitro*. Arthritis Rheum 1987; 30: 530–540.
18. Delaisse JM, Eeckhout Y, Vaes G. Bone resorbing agents affect production and distribution of procollagenase in bone tissue. Endocrinology 1988; 123: 264–276.
19. Einhorn TA, Majeska RJ. Neutral proteases in regenerating bone. Clin Orthop 1991; 262: 286–297.
20. Everts V, Delaisse JM, Korper W, Niehof A, Vaes G, Beertsen W. Degradation of collagen in bone resorbing compartment underlying the osteoclast involves both cysteine-proteinases and matrix metalloproteinases. J Cell Physiol 1992; 150: 221–231.
21. Hill PA, Reynolds JJ, Meikle MC. Inhibition of stimulated bone resorption *in vitro* by TIMP-1 and TIMP2. Biochim Biophys Acta Mole Cell Res 1993; 1177: 71–74.
22. Leloup G, Delaisse JM, Vaes G. Relationship of the plasminogen activator/plasmin cascade to osteoclast invasion and mineral resorption in explanted fetal metatarsal bones. J Bone Miner Res 1994; 9: 891–902.

23. Zheng MH, Wood DJ, Papadimitriou JM, Nicholson GC. Evidence that protein kinase-A, calcium-calmodulin kinase and cytoskeletal proteins are involved in osteoclast retraction induced by calcitonin. Exper Mole Pathol 1992; 57: 105–115.
24. Baxter E, Fraser JRE, Harris GS, Martin TJ. Stimulation of glycosaminoglycan synthesis by thyrocalcitonin preparations. Med J Australia 1968; 10: 216–217.
25. Suzuki F, Yoneda T, Shinomura Y. Calcitonin and parathyroid hormone stimulation of acid mucopolysaccharide synthesis in cultured chondrocytes isolated from growth cartilage. FEBS Letters 1976; 70: 155–158.
26. Kato Y, Nasu N, Takase T, Daikuhara Y, Suzuki F. A serum-free medium supplemented with multiplication-stimulating activity (MSA) supports both proliferation and differentiation of chondrocytes in primary culture. Exper Cell Res 1980; 125: 167–174.
27. Burch WM. Calcitonin stimulates growth and maturation of embryonic chick pelvic cartilage *in vitro*. Endocrinology 1984; 114: 1196–1202.
28. Havelka S, Babicky A, Musilova J, Rohozkova D, Tesarek B. Effect of osteotropic hormones on cartilage metabolism. Horm Metab Res 1979; 11: 83–84.
29. Franchimont P, Bassler C, Henrotin Y, Gysen P, Bassler R. Effects of human and salmon calcitonin on human articular chondrocytes cultivated in clusters. J Clin End Metab 1989; 69: 259–266.
30. Badurski JE, Schwamm W, Popko J, Zimnoch L, Rogowski F, Pawlica J. Chondroprotective action of salmon calcitonin in experimental arthropathies. Lab Invest 1991; 49: 27–34.
31. Colombo C, Butler M, Hickman L, Selwyn M, Chart J, Steinetz B. A new model of osteoarthritis in rabbits. II. Evaluation of anti-osteoarthritic effects of selected antirheumatic drug administered systemically. Arthritis Rheum 1983; 26: 1132–1139.
32. Twining SS. Fluorescein isothiocyanate-labelled casein assay for proteolytic enzymes. Analytical Biochem 1984; 143: 30–34.
33. Smith PK, Krohn RI, Hermanson GT, Mallia AK, Gartner FH, Provenzano MD, Fujimoto EK, Goeke NM, Olson BJ, Klenk DC. The bicinchoninic acid solution for protein determination. Anal biochem 1985; 150: 76–85.
34. Morris JO, Blatts S, Powell JR, Strickland DK, Castellino FJ. Role of lysine binding region in the kinetic properties of human plasmin. Biochemistry 1981; 20: 4811–6.
35. Qwarnstrom EE, Page RC, Gillis S, Dower SK. Binding, internalization and intracellular localization of interleukin-1β in human diploid fibroblast. J Biol Chem 1988; 263: 8261–9.
36. Aumailley M, Mann K, Von der Mark H, Timpl R. Cell attachment properties of collagen VI and Arg-Gly-Asp dependent binding to its α2(VI) and α3(VI) chains. Exp Cell Res 1989; 181: 463–74.
37. Vignon E, Mathieu P, Louisot P, Richard M. *In vitro* effect of nonsteroidal antiinflammatory drugs on proteoglycanase and collagenase activity in human osteoarthritic cartilage. Arthritis Rheum 1991; 34: 1332–5.
38. Werb Z, Mainardi CL, Vater CA, Harris ED. Endogenous activation of latent collagenase by rheumatoid synovial cells. N Engl J Med 1977; 296: 1017–23.
39. Baca S, Altman RD, Dean DD. The effect of synthetic salmon calcitonin (SCT) on rabbit articular cartilage. Arthritis Rheum 1991; 34 (Suppl), S104, B38.
40. Suffys P, Van Roy F, Fiers W. Tumor necrosis factor and interleukin 1 activate phospholipase in rat chondrocytes. FEBS Lett 1988; 232: 24–8.
41. Ceserani R, Colombo M, Olgiati VR, Pecile A. Calcitonin and prostaglandin system. Life Sci 1979; 25: 1851–6.
42. Havelka S, Hurych J. *In vitro* effects of calcitonin on inflammation. Horm Metab Res 1980; 12: 226–7.
43. Strettle RJ, Bates RFL, Buckley BA. Evidence for a direct anti-inflammatory action of calcitonin: inhibition of histamine induced mouse pinnal oedema by porcine calcitonin. J Pharm Pharmacol 1980; 32: 192–5.
44. Aida S, Okoawa-Takatsuji M, Aotsuka S, Shimoji K, Yokohari R. Calcitonin inhibits production of immunoglobulins, rheumatoid factor and interleukin-1 by mononuclear cells from patients with rheumatoid arthritis. Ann Rheum Dis 1994; 53: 247–9.
45. Pennypacker JP, Wassell JR, Yamada KM, Pratt RM. The influence of an adhesive cell surface protein on chondrogenic expression *in vitro*. Exp Cell Res 1979; 121: 411–5.
46. Genari G, Gonnelli S, Agnusdei D, Civitelli R. Biological activity of different calcitonins in men. Exp Gerontology 1990; 25: 339–47.
47. Nuesch E, Schmidt R. Comparaison de la pharmacocinétique des calcitonines. In: A. Pecile ed. La Calcitonine en 1980. Amsterdam: Excerpta Medica 1981, 378–91.