Calcif Tissue Int (1992) 50:11–13

Calcified Tissue International

Effect of Calcitonin on Bone Histomorphometry and Bone Metabolism in Rheumatoid Arthritis

Heikki Kröger, Ilkka Arnala, and Esko M. Alhava

Department of Surgery, Kuopio University Hospital, SF-70211 Kuopio, Finland

Received March 15, 1991, and in revised form June 5, 1991

Summary: Twenty-four women (mean age ± SD 49 ± 13 years) with classical or definite rheumatoid arthritis (disease duration 15 ± 8 years) were treated with synthetic salmon calcitonin (SCT) nasal spray 200 IU three times a week for 3 months. Bone biopsies from the iliac crest were taken before and after SCT treatment. Histomorphometrical quantification of undecalcified bone sections was made using the manual point-counting method. SCT decreased the resorption surface of trabecular bone (ES/BS) significantly ($P < 0.001$). There was also a significant increase ($P < 0.05$) in trabecular bone volume (BV/TV) after 3 months of treatment, whereas no statistically significant changes were found in osteoid parameters. There were no significant changes in biochemical analyses of bone metabolism. We conclude that SCT might be useful in the prevention of bone loss in RA.

Key words: Calcitonin – Rheumatoid arthritis – Bone histomorphometry.

The best known consequences of rheumatoid arthritis (RA) are inflammation of the synovium and destruction of cartilage. Moreover, RA is characterized by both periarticular and generalized osteoporosis. Periarticular bone loss, which is an early radiological finding in RA [1], occurs before there is any significant damage to the articular surface [2]. Periarticular osteoporosis may be due to immobilization of the affected joints or to local inflammatory factors.

Several studies in RA patients have demonstrated generalized osteoporosis of the axial and appendicular skeleton [3–5]. Many potential factors may contribute to generalized bone loss: the patient's age, sex, menopausal status, reduced mobility, concomitant corticosteroid or other drug therapy, and the disease process itself [5, 6]. In fact, osteoporosis in RA is a multifactorial process.

Considering the inhibitory effect of calcitonin on bone resorption [7, 8] and its efficacy in the treatment of other bone diseases [9–12], calcitonin might be effective in the treatment of osteoporosis associated with RA. The main purpose of the present study was to examine the effect of calcitonin, administered intranasally, on bone histomorphometry in patients with RA. We also report the effect of calcitonin on biochemical parameters of bone metabolism.

Offprint requests to: H. Kröger

Patients and Methods

Patients

Twenty-four women (mean age 49 years, range 29–64) with classical or definite RA (according to ARA criteria) were studied. Twelve of these patients were postmenopausal. Patients with a history of gastrointestinal disease or surgery, endocrine disease, chronic renal disease, liver disease, or other disease known to affect bone metabolism were excluded from the study. One patient was receiving postmenopausal estrogen therapy. The mean duration of the disease was 15 years (range 4–41). All were taking nonsteroidal antiinflammatory drugs and most were receiving specific antiinflammatory drug therapy (gold compounds, penicillamine, antimalarials or salazopyrine (sulphasalazine). Four patients had received low-dose glucocorticoids during the past disease course (<7.5 mg prednisolon/day, duration of the treatment <1 years). None of the patients had received systemic steroid therapy for at least 8 months, however. Twenty women had never been treated with systemic glucocorticoids. Disability was assessed by the method of Steinbroker et al. [13]. Clinical details of the patients are shown in Table 1.

The patients were treated with synthetic salmon calcitonin (SCT) (Miacalcic Nasal, Sandoz Pharma Ltd, Basel, Switzerland) administered intranasally for 3 months. The dosage of SCT was 200 IU three times a week.

Bone Histomorphometry

Bone biopsy specimens were obtained by the vertical approach 2–3 cm behind the anterior superior iliac spine. A trephine with an internal diameter of 5 mm was used. A biopsy was taken from each side of the pelvis before and after treatment. Undecalcified sections (5 μm) were stained with Masson-Goldner trichrome stain, and 30–90 fields were measured in two to six consecutive sections with a Merz graticule using a magnification of ×100. Trabecular bone was measured under the cortex and subcortical bone. All histomorphometrical measurements were performed without knowledge of the clinical details by the same observer. The following indices were obtained by measurements or calculation: trabecular bone volume (BV/TV), osteoid volume (OV/BV), osteoid surface (OS/BS), eroded surface (ES/BS), and osteoid seam thickness index (TIOS; 100 * OV/BV:OS/BS).

Tetracycline double labeling was performed in 19 patients. The labeling protocol consisted of giving oral oxytetracycline 250 mg q.i.d. for 1 day, no medication for 5 days, and again oxytetracycline 250 mg q.i.d. for 1 day, followed by a biopsy 5 days later [14]. Mineral apposition rate (MAR) was measured from unstained sections according to Frost [15] using a magnification of ×625.

Biochemical Determinations

Serum parameters were obtained after the subjects had fasted over-

Table 1. Clinical details of the RA patients receiving calcitonin treatment (N = 24)

Mean age (SD)	49 (13) years
Mean weight (SD)	65 (13) kg
Mean height (SD)	161 (7) cm
Pre-/postmenopausal	12:12
Mean duration of disease (SD)	15 (8) years
Functional class	
II	14 patients
III	10 patients

Table 2. Bone histomorphometric data (mean with SD) of 22 RA patients during calcitonin treatment

	0 month	3 months	Significance
BV/TV (%)	15.0 (3.3)	16.4 (3.2)	$P < 0.05$
OV/BV (%)	2.2 (1.2)	1.9 (1.1)	NS
OS/BS (%)	19.3 (9.2)	17.2 (5.6)	NS
ES/BS (%)	3.3 (1.0)	1.5 (0.5)	$P < 0.001$
TIOS	11.1 (3.7)	10.8 (3.6)	NS
MAR (μm/day)[a]	0.45 (0.07)	0.46 (0.06)	NS

[a] N = 19

night. Serum calcium (S-Ca), phosphate (S-Pi), alkaline phosphatase (S-ALP), alanine transaminase (S-ALAT), aspartate transaminase (S-ASAT), glutamyl transferase (S-GT), total protein (S-Prot), albumin (S-Alb), and creatinine (S-Crea) were determined using standard laboratory methods. S-Ca values were corrected to serum albumin values using the equation S-Ca(corr) = S-Ca + (44 − S-Alb (g/liter) * 0.019. The erythrocyte sedimentation rate (ESR) and C-reactive protein (S-CRP) were also measured. Diurnal calcium (dU-Ca) and creatinine (dU-Crea) excretion were determined with no dietary restrictions. Serum 25-hydroxycholecalciferol (S-25OHD) and serum 1,25-dihydroxycholecalciferol [1,25$(OH)_2$D] were measured by specific protein binding methods after chromatographic purification [16]. Serum bone Gla protein (S-BGP) was determined by radioimmunoassay using a commercial kit manufactured by CIS (Compagnie ORIS Industrie S.A., France), and each determination was carried out in duplicate. The intraassay coefficient of variation was 6.9% and the interassay variation was 9.1% [17]. The biochemical determinations were carried out before and after calcitonin treatment.

Pain Index and Morning Stiffness

By using the visual analoque scale (VAS) [18], the pain index was evaluated before and after calcitonin treatment. Patients were also asked about duration of morning stiffness.

Statistical Analysis

Data were analyzed using Student's *t* test for paired data. The results are expressed as means ± SD.

Results

Bone histomorphometric data are available from 22 patients (Table 2). After 3 months of treatment with SCT, BV/TV was increased ($P < 0.05$). Osteoid parameters decreased but the changes were not statistically significant. ES/BS decreased significantly during SCT trial ($P < 0.001$). SCT treatment had no effect on MAR. No statistically significant changes were found in the biochemical parameters of calcium metabolism (Table 3). However, there were significant decreases in S-ASAT, S-ALAT, and S-GT concentrations after SCT trial.

The pain index decreased slightly, but the change did not reach statistical significance (4.6 ± 2.0 versus 4.2 ± 2.0; NS). Morning stiffness did not change during treatment (1.7 ± 1.3 hours versus 1.6 ± 1.5 hours; NS).

Discussion

Several studies have shown that patients with RA have decreased bone mass at various skeletal sites [4, 6, 19, 20].

Table 3. Biochemical parameters (mean with SD) among RA patients (N = 24) during calcitonin treatment

	0 month	3 months
S-Ca (mmol/liter)	2.41 (0.11)	2.38 (0.13)
S-Pi (mmol/liter)	1.02 (0.16)	1.07 (0.17)
S-Alb (g/liter)	39 (4)	38 (5)
S-Prot (g/liter)	73 (5)	70 (5)
S-Ca_{corr}(mmol/liter)	2.50 (0.11)	2.50 (0.14)
S-Crea (μmol/liter)	69 (10)	70 (10)
S-ALP (U/liter)	166 (49)	160 (59)
S-ASAT (U/liter)	23 (9)	19 (4)[a]
S-ALAT (U/liter)	21 (14)	16 (9)[a]
S-GT (U/liter)	23 (19)	21 (18)[a]
S-BGP (ng/ml)	8.0 (1.9)	7.4 (2.6)
S-25(OH)D (nmol/liter)	25 (18)	22 (13)
S-1,25$(OH)_2$D (pmol/liter)	47 (15)	46 (17)
dU-Ca (mmol)	3.1 (1.7)	2.8 (1.7)
dU-Crea (mmol)	8.2 (3.0)	7.8 (1.9)
dU-Ca/dU-Crea	0.39 (0.19)	0.36 (0.21)

[a] $P < 0.01$

SCT has been used in the prevention and treatment of postmenopausal osteoporosis. A number of studies have suggested its efficacy in this disorder [9–12]. So far there has been only one report concerning calcitonin treatment for RA. Dequeker et al. [21] used bone densitometry and found significant difference in bone mineral density at the spine and distal radius between calcitonin- and placebo-treated groups. Bone histomorphometry has not been used in previous studies to assess the therapeutic effects of calcitonin in RA.

In the present study, SCT (200 IU), given intranasally three times a week, decreased bone resorption significantly (55%) after 3 months of treatment. The inhibition of bone resorption in our patients is in accordance with the known action of calcitonin [8]. There was also an increase in BV/TV, which could be caused by the decrease in bone resorption. The variability between adjacent or consecutive samples at the iliac crest is considerably high, however, and this must be taken into account in longitudinal studies [22]. However, the increase of TBV was 9.3%, which is higher than the possible intersample variation for 20 cases (7%) reported by Chavassieux et al. [22]. There were no significant changes in osteoid parameters during treatment, leading to the assumption that bone formation is not affected by SCT treatment.

The studies of S-BGP, a proposed marker of bone formation, have revealed discrepant findings in RA [23–25]. In the present study, S-BGP was not altered significantly during SCT treatment. This was presumed, because calcitonin has hardly any direct effect on osteoblasts. However, there could have been a general decrease in bone metabolism ow-

ing to coupling. Surprisingly, significant decreases in S-ALAT, S-ASAT, and S-GT determinations were found. This might have resulted from decreased use of NSAIDs. Unfortunately, this cannot be proved, because the use of nonsteroidal antiinflammatory drugs (NSAIDs) during SCT treatment was not evaluated. The decrease of liver enzymes during calcitonin treatment has not been reported previously. So far this finding remains unexplained.

The analgesic effect of calcitonin has been demonstrated in previous studies [26, 27]. No significant changes, however, could be found in pain indexes or in morning stiffness in our patients after treatment. One possible explanation is that calcitonin has no effect on the activity of the disease, which can change during treatment. Calcitonin given intranasally proved to be a well-tolerated drug. Only 2 patients had side effects (nausea) in the beginning of treatment; it disappeared in a week without having to lower the dose.

Using histomorphometry, we demonstrated for the first time the expected ability of intranasal SCT to inhibit bone resorption in RA patients. Our results are supported by several previous studies of postmenopausal osteoporosis [10, 12, 28]. We suggest that SCT is a useful treatment for bone loss associated with RA. The treatment period of the present study was, however, only 3 months; further studies of longer treatment periods might be needed.

References

1. Bywaters EGL (1960) The early radiological signs of rheumatoid arthritis. Bull Rheum Dis 11:231–234
2. Soila P (1963) The causal relations of rheumatoid disintegration of juxta-articular bone trabeculae. Acta Rheum Scand 9:231–236
3. Duncan H, Frost HM, Villaneuva AR, Sigler JW (1965) The osteoporosis of rheumatoid arthritis. Arthritis Rheum 8:943–954
4. Reid DM, Kennedy NSJ, Smith MA, Tothill P, Nuki G (1982) Total body calcium in rheumatoid arthritis: effects of disease activity and corticosteroid therapy. Br Med J 285:330–332
5. Sambrook PN, Eisman JA, Champion GD, Yeates MG, Pocock NA, Eberl S (1987) Determinants of axial bone loss in rheumatoid arthritis. Arthritis Rheum 30:721–728
6. Als OS, Gotfredsen A, Riis BJ, Christiansen C (1985) Are disease duration and degree of functional impairment determinants of bone loss in rheumatoid arthritis? Ann Rheum Dis 44:406–411
7. Friedman J, Raisz LG (1965) Thyrocalcitonin, inhibitor of bone resorption in tissue culture. Science 150:1465–1467
8. Singer FR, Melvin KEW, Mills BG (1976) Acute effects of calcitonin on osteoclasts in man. Clin Endocrinol 5:333–340
9. Gruber HE, Ivey JL, Baylink DJ, Matthews M, Nelp BP, Sisom K, Chesnut CH III (1984) Long-term calcitonin therapy in postmenopausal osteoporosis. Metabolism 33:295–303
10. Mazzuoli GF, Passeri M, Gennari C, Minisola S. Antonelli R, Valtorta C, Palummeri E, Cervellin GF, Gonnelli S, Francini (1986) Effect of salmon calcitonin in postmenopausal osteoporosis: a controlled double-blind clinical study. Calcif Tissue Int 38:3–8
11. Reginster JY, Denis D, Albert A, Deroisy R, Lecart MP, Fontaine MA, Lambelin P, Franchimont P (1987) 1-year controlled randomized trial of prevention of early postmenopausal bone loss by intranasal calcitonin. Lancet ii:1481–1483
12. Overgaard K, Riis BJ, Christiansen CJ, Hansen MA (1989) Effect of calcitonin given intranasally on early postmenopausal bone loss. Br Med J 299:477–479
13. Steinbrocker O, Traeger CH, Batterman RC (1949) Therapeutic criteria in rheumatoid arthritis. JAMA 140:659–662
14. Frost HM (1983) Bone histomorphometry: choice of marking agent and labeling schedule. In: Recker RR (ed) Bone histomorphometry: techniques and interpretation. CRC Press, Boca Raton, p 37
15. Frost HM (1969) Tetracycline-based histological analysis of bone remodeling. Calcif Tissue Res 3:211–237
16. Parviainen MT, Savolainen KE, Korhonen PH, Alhava EM, Visakorpi JK (1981) An improved method for routine determination of vitamin D and its hydroxylated metabolites in serum from children and adults. Clin Chim Acta 114:233–247
17. Kröger H, Penttilä I (1990) Osteocalcin in serum of healthy Finnish women determined by radioimmunoassay—effects of menopause on its concentration. Scand J Clin Lab Invest 50(suppl 202):187
18. Huskisson EC (1974) Measurement of pain. Lancet ii:1127–1131
19. Kennedy AC, Lindsay R (1977) Bone involvement in rheumatoid arthritis. Clin Rheum Dis 3:403–420
20. Oka M, Rekonen A, Kuikka J, Anttinen J (1975) Bone mineral density in rheumatoid arthritis measured by the gamma transmission method. Scand J Rheumatol 4:28–32
21. Dequeker J, Geusens P, Verstraeten A, Sileghem A (1990) Effects of nasal calcitonin on pain, bone erosion and bone loss in rheumatoid arthritis (abstract). In: Overgaard K, Christiansen C (eds) 3rd Int Symp on Osteoporosis. Handelstrykkeriet Aalborg ApS, Aalborg, p 128
22. Chavassieux PM, Arlot ME, Meunier PJ (1985) Intersample variation in bone histomorphometry: comparison between parameter values measured on two contiguous transiliac bone biopsies. Calcif Tissue Int 37:345–350
23. Als OS, Riis BJ, Gotfredsen A, Christiansen C, Deftos LJ (1986) Biochemical markers of bone turnover in rheumatoid arthritis. Acta Med Scand 219:209–213
24. Gevers G, Devos P, De Roo M, Dequeker J (1986) Increased levels of osteocalcin (serum bone gla-protein) in rheumatoid arthritis. Br J Rheumatol 25:260–262
25. Pietschmann P, Machold KP, Wolosczuk W, Smolen JS (1989) Serum osteocalcin concentrations in patients with rheumatoid arthritis. Ann Rheum Dis 48:654–657
26. MacIntyre I, Evans IMA, Hobitz HHG, Joplin GF, Stevenson JC (1980) Chemistry, physiology, and therapeutic applications of calcitonin. Arthritis Rheum 23:1139–1147
27. Gennari C (1983) Clinical aspects of calcitonin in pain. Triangle 22:157–163
28. MacIntyre I, Stevenson JC, Whitehead MJ, Wimalawansa SJ, Banks LM, Healy MJR (1988) Calcitonin for prevention of postmenopausal bone loss. Lancet i:900–902