CLINICAL STUDIES

A Randomized Trial of Nasal Spray Salmon Calcitonin in Postmenopausal Women with Established Osteoporosis: the Prevent Recurrence of Osteoporotic Fractures Study

Charles H. Chesnut, III, MD, Stuart Silverman, MD, Kim Andriano, PhD, Harry Genant, MD, Alberto Gimona, MD, Steven Harris, MD, Douglas Kiel, MD, Meryl LeBoff, MD, Michael Maricic, MD, Paul Miller, MD, Caje Moniz, MD, Munro Peacock, MD, Peter Richardson, MRCP, Nelson Watts, MD, David Baylink, MD, for the PROOF Study Group

PURPOSE: We conducted a 5-year, double-blind, randomized, placebo-controlled study to determine whether salmon calcitonin nasal spray reduced the risk of new vertebral fractures in postmenopausal women with osteoporosis.

SUBJECTS AND METHODS: A total of 1,255 postmenopausal women with established osteoporosis were randomly assigned to receive salmon calcitonin nasal spray (100, 200, or 400 IU) or placebo daily. All participants received elemental calcium (1,000 mg) and vitamin D (400 IU) daily. Vertebral fractures were assessed with lateral radiographs of the spine. The primary efficacy endpoint was the risk of new vertebral fractures in the salmon calcitonin nasal spray 200-IU group compared with the placebo group.

RESULTS: During 5 years, 1,108 participants had at least one follow-up radiograph. A total of 783 women completed 3 years of treatment, and 511 completed 5 years. The 200-IU dose of salmon calcitonin nasal spray significantly reduced the risk of new vertebral fractures by 33% compared with placebo [200 IU: 51 of 287, placebo: 70 of 270, relative risk (RR) = 0.67, 95% confidence interval (CI): 0.47– to 0.97, $P = 0.03$]. In the 817 women with one to five prevalent vertebral fractures at enrollment, the risk was reduced by 36% (RR = 0.64, 95% CI: 0.43– to 0.96, $P = 0.03$). The reductions in vertebral fractures in the 100-IU (RR = 0.85, 95% CI: 0.60– to 1.21) and the 400-IU (RR = 0.84, 95% CI: 0.59– to 1.18) groups were not significantly different from placebo. Lumbar spine bone mineral density increased significantly from baseline (1% to 1.5%, $P < 0.01$) in all active treatment groups. Bone turnover was inhibited, as shown by suppression of serum type-I collagen cross-linked telopeptide (C-telopeptide) by 12% in the 200-IU group ($P < 0.01$) and by 14% in the 400-IU group ($P < 0.01$) as compared with placebo.

CONCLUSION: Salmon calcitonin nasal spray at a dose of 200 IU daily significantly reduces the risk of new vertebral fractures in postmenopausal women with osteoporosis. **Am J Med. 2000;109:267–276.** ©2000 by Excerpta Medica, Inc.

Calcitonin, a physiologic endogenous inhibitor of bone resorption, decreases osteoclast formation (1,2), osteoclast attachment (2,3), and bone resorption in organ culture and animal models (1,4,5).

Thus, treatment with calcitonin may be beneficial in diseases associated with increased bone resorption, such as postmenopausal osteoporosis (6). Several studies (7–21) have suggested that salmon calcitonin, administered as an injection or a nasal spray, is safe and can stabilize or increase bone mineral density. However, although bone appears to be of normal quality after salmon calcitonin treatment—in terms of mechanical performance, material density, and patterns of collagen birefringence (22–26)—the efficacy of salmon calcitonin in reducing fractures remains to be determined in a large randomized, controlled trial. Previous studies indicating fracture reduction at the spine and hip have been retrospective (27) or, if prospective, involved small numbers of participants (13–15,17–20). Therefore, we conducted a 5-year, multicenter clinical trial [the Prevent Recurrence of Osteoporotic Fractures (PROOF) study] to determine the long-term efficacy and safety of salmon calcitonin nasal spray in the prevention of vertebral fractures in postmenopausal women with osteoporosis.

MATERIAL AND METHODS

This double-blind, placebo-controlled trial was conducted in 42 centers in the United States and five centers in the United Kingdom. A total of 1,255 women were enrolled between February 1991 and July 1993.

Study Participants

White, Asian, or Hispanic women were eligible to participate if they were postmenopausal for at least 1 year and had one to five prevalent thoracic or lumbar vertebral compression fractures as evaluated at the study center, lumbar spine bone mineral density at least 2 SD below normal for normal women age 30 years, and no history of hip fracture. Women with a history of diseases, conditions, or chronic usage of medications (eg, corticosteroids) that could affect bone metabolism or bone mass measurements were excluded, as were those who had been treated with calcitonin, estrogens, or fluorides within 3 months of study entry, continuous bisphosphonates for at least 3 months within 24 months, or cyclical bisphosphonates within 18 months. The study was performed in accordance with the US Code of Federal Regulations dealing with clinical studies and the Declaration of Helsinki concerning medical research in humans. Women provided informed consent before any study-specific procedure was performed. Institutional Review Boards/Ethics Committees approved the protocol at each center.

Treatment Protocols/Follow-up Studies

Participants were assigned to receive salmon calcitonin nasal spray at a dose of 100, 200, or 400 IU (Miacalcin Nasal Spray; Novartis Pharmaceuticals, East Hanover, New Jersey) or placebo nasal spray, using a computer-generated randomization list. The randomization code was stratified by center using a permuted block design with a block size of eight. The nasal spray containers looked identical and had similar labels. All participants received two 500-mg OS-CAL tablets (1,000 mg oral calcium) and one Centrum tablet daily (400 IU vitamin D) to ensure a minimum daily intake of 1,500 mg of calcium and adequate vitamin D daily intake. Evaluations were performed at months 1, 3, 6, 9, 12, and every 6 months thereafter up to month 60 or in case of participant discontinuation. Adherence was estimated by counting used and unused bottles of study medication. Spinal radiographs; lumbar spine and hip bone mineral density; serum type-I collagen cross-linked C-telopeptide, bone-specific alkaline phosphatase, and osteocalcin levels; urinary type-1 collagen cross-linked N-telopeptide levels; and calcitonin binding antibodies were assessed every year. Participants were monitored closely for medication safety and tolerability throughout the study.

Analytical Procedures

Lateral thoracic and lumbar radiographs were evaluated qualitatively at each study center before enrollment, and 1,255 women were enrolled based on the initial radiograph report at the study site. Subsequently, all baseline and follow-up lateral thoracic and lumbar radiographs were analyzed at the University of California, San Francisco, using a combined quantitative and semiquantitative method (28,29). Based on this review, 269 women who were initially determined by the study site principal investigator to meet the criteria of one to five vertebral fractures were found to have only a mild compression fracture that did not meet the enrollment criteria. An additional 65 women were found to have more than five vertebral fractures. However, all enrolled participants were allowed to continue in the study. Prevalent fractures were defined as a 3 or greater SD reduction in any height ratio (vs normative data) by quantitative morphometry and a fracture grade 1 or greater (where grade 0 is "no fracture" and grade 3 is "severe fracture") using a semiquantitative evaluation. Two independent radiologists made the evaluation, with adjudication by a third radiologist in the event of discrepant quantitative and semiquantitative results. Incident fractures were defined as a 20% or greater and greater than 4-mm decrease in any vertebral height (vs previous radiograph) by quantitative morphometry, as well as a change in the fracture grade from 0 to 1 or greater by semiquantitative evaluation, with adjudication in discrepant cases as outlined above (28,29). Nonvertebral fractures were recorded and verified by hospital records. Participants were not withdrawn from the study when they had a fracture.

Bone mineral density at the lumbar spine and hip (femoral neck, greater trochanter, and Ward's triangle) was evaluated by dual x-ray absorptiometry (DXA) using Hologic (QDR 1000, 1500, 2000; Waltham, Massachusetts), Lunar (DPXL-DPXIQ; Madison, Wisconsin), or Norland (XR26-XR36; White Plains, New York) densitometers. Lumbar vertebras with prevalent or incident fractures at L1 to L4 were not included in the bone mineral density measurements. A quality-control procedure to enable pooling of the data from the different centers and densitometers (including scanning of a phantom by all centers) was conducted at the University of California, San Francisco (30,31). The longitudinal in vitro precision error for lumbar spine bone mineral density measurements ranged from 0.3% to 2.0% over 5 years. Investigators were not blinded to the bone mineral density measurements.

Serum samples for C-telopeptide, bone-specific alkaline phosphatase, and osteocalcin levels were obtained primarily in the morning hours and assessed centrally at the Jerry L. Pettis Veterans Affairs (VA) Medical Center (Loma Linda, California). Samples were frozen at −70° C

after collection. Bone-specific alkaline phosphatase was assessed by the method of Farley et al (32) using a heat-inactivation assay with an interassay variation of 6.3%. Osteocalcin was assessed with the n-terminus, mid-molecule assay (33,34); the interassay variation was 7.7%. For C-telopeptide (CrossLaps; Osteometer Biotech, Herlev, Denmark), samples were batch-assayed at the end of the study to ensure that samples from any given participant were assayed together to minimize interassay variation. The analytical interassay precision was ± 8.1%.

The urinary N-telopeptide/creatinine ratio was also assessed centrally at the Jerry L. Pettis VA Medical Center. Urine samples were frozen at −20° C. The urinary N-telopeptide samples were analyzed in multiple batches, and it was discovered that there was a decrease in recovery of urinary creatinine over time, perhaps resulting from the relatively high storage temperature; thus, the N-telopeptide results are not reported.

Salmon calcitonin antibody titers were determined centrally by radioimmunoassay (35) at the Cedars-Sinai Medical Center in California.

Statistical Analyses

The primary analysis for the incident vertebral fracture endpoint was an intention-to-treat analysis among all participants with at least one follow-up radiograph. Secondary analyses were performed among participants with one to five prevalent vertebral fractures at enrollment (as per protocol) and among those who received the study drug for at least 3 years or who had a fracture during the first 3 years of treatment (3-year valid completer analysis). The 3-year duration is the minimum length required by regulatory guidelines to demonstrate a therapeutic effect on vertebral fractures. The original study design was intended to compare the risk of new vertebral fractures between the placebo group and each of the active treatment groups. After the approval of salmon calcitonin nasal spray 200 IU in the United States for the treatment of osteoporosis, and the issuing of the new Food and Drug Administration (FDA) guidelines, the protocol was amended in 1996, such that the primary statistical assessment was changed to be the pairwise comparison of 200 IU versus placebo using statistical life-table methods. The study had a power of 80% to show a 50% reduction in the risk of new vertebral fractures, on the assumption that 20% of participants would have a fracture in the placebo group compared with 10% in the salmon calcitonin nasal spray 200-IU group. The study was not designed to have power to discriminate between doses. All reported *P* values are two –sided, and treatment contrasts are presented with their 95% confidence intervals (CI).

Descriptive statistics, one-way analysis of variance (ANOVA), F tests, and chi-squared tests were used to compare the treatment groups at baseline. Time to first new fracture or time to fracture after administration of study drug was analyzed primarily by life-table methods using the proportional hazards model with treatment as a variable (36). Relative risks were estimated as hazard ratios. Kaplan-Meier estimates and plots provided descriptive measures of fracture rates. Data about multiple new fractures were analyzed using the Wilcoxon rank sum test (chi-squared approximation). The effects of treatment of the risk of developing two or more new vertebral fractures were estimated as odds ratios from logistic regression models. Life-table methods using proportional hazards models were also used for nonvertebral fractures.

For bone mineral density and markers of bone metabolism among women who withdrew from the trial prematurely, the last value was carried forward to subsequent visits. Descriptive statistics were calculated on percent change from baseline for bone mineral density and serum C-telopeptide and osteocalcin levels at each evaluable time point. Descriptive statistics for bone-specific alkaline phosphatase levels were calculated as change from baseline. Women who developed calcitonin antibodies above 1,000 at any time were tabulated.

ANOVA or chi-square tests were used to compare groups. Serum C-telopeptide levels were skewed, and nonparametric statistics were used to compare groups. The overall effect on serum C-telopeptide levels (baseline to year 5) was evaluated by comparing least square means of different groups by the Proc Mixed output procedure (37) (from the Statistical Analysis System, Cary, North Carolina), which provides a descriptive measure of treatment effect compared with placebo during the entire study period by using the observed correlations structures within the participants' longitudinal data (also known as "repeated measures" data).

RESULTS

More than 3,500 women were screened for study participation, of whom 1,255 were randomly assigned to either placebo (n = 311), salmon calcitonin nasal spray 100 IU (n = 316), 200 IU (n = 316), or 400 IU (n = 312) (Table 1). After adjudication of baseline spine radiographs, 910 women had one to five prevalent vertebral fractures (as specified by the protocol), 269 had no vertebral fractures, and 65 had more than five fractures. Spinal radiographs could not be evaluated in 11 women, who were excluded from all analyses.

Baseline characteristics of the participants, including age, years since menopause, body mass index, number of prevalent fractures, lumbar spine bone mineral density, calcium intake, smoking history, and serum C-telopeptide levels, were similar among the groups (Table 2). More than 90% of women were more than 75% adherent to treatment during the time they were in the trial. Fifty-nine percent of the participants (744 of the 1,255 who

Table 1. Participation and Reasons for Withdrawal, by Randomization Group

| | | Treatment Group | | |
| | | Nasal Spray Salmon Calcitonin | | |
	Placebo (n = 311)	100 IU (n = 316)	200 IU (n = 316)	400 IU (n = 312)
Completed 3 years	190	189	204	200
Completed study	128	124	132	127
Withdrawals	183	192	184	185
Drug-related adverse effect	21	21	19	31
Adverse effect or illness not related to study drug	56	50	51	57
Lack of cooperation	23	16	23	14
Protocol violation	10	11	14	12
Ineffective study drug	25	25	15	17
Other*	48	71	62	54

*Other reasons for discontinuation include lost to follow-up, consent withdrawn, and switched to another therapy. No statistically significant differences were observed for any reason for discontinuation between any of the treatment groups and placebo.

were enrolled) withdrew from the study prematurely. Rates of discontinuation were similar in all of the dosage groups (Table 1); for example, 4.4% of participants in the salmon calcitonin nasal spray groups and 3.3% of participants in the placebo group discontinued because of nasal events. To determine whether the relatively high rate of early discontinuation led to selection bias, the baseline characteristics of the participants still at risk of a first new vertebral fracture (at years 3 and 4) were compared among groups; no statistically significant differences were observed. To determine if nonresponders had discontinued selectively in any treatment group, response to treatment (as lumbar spine bone mineral density and serum C-telopeptide levels) was compared among groups in participants who discontinued before years 3 and 4 and who were still at risk of first new vertebral fracture. Although there were no significant differences in suppression of serum C-telopeptide levels, participants who dis-

Table 2. Baseline Demographic Characteristics of Enrolled Women

| | | Treatment Group | | |
| | | Nasal Spray Salmon Calcitonin | | |
	Placebo (n = 311)	100 IU (n = 316)	200 IU (n = 316)	400 IU (n = 312)
		Number (Percent) or Mean ± SD		
Age (years)				
<65	99 (32)	100 (32)	84 (27)	95 (30)
65–74	148 (48)	149 (47)	153 (48)	166 (53)
≥75	64 (21)	67 (21)	79 (25)	51 (16)
Age (years)	68.2 ± 7.7	68.2 ± 7.8	69.0 ± 8.1	67.9 ± 6.9
Years since menopause	22.0 ± 9.4	22.2 ± 9.2	23.0 ± 10.0	21.9 ± 8.4
Body mass index (kg/m^2)	24.7 ± 3.9	24.7 ± 3.8	25.0 ± 3.7	24.9 ± 3.6
Vertebral fractures at baseline*				
0	64 (21)	79 (25)	67 (21)	59 (19)
1–5	232 (75)	223 (71)	224 (71)	231 (74)
>5	13 (4)	10 (3)	21 (7)	21 (7)
Lumbar spine bone mineral density (g/cm^2)	0.85 ± 0.12	0.84 ± 0.11	0.85 ± 0.11	0.84 ± 0.12
Calcium intake (mg/day)†	979 ± 592	907 ± 563	911 ± 452	874 ± 480
Current smokers	47 (15)	51 (16)	44 (14)	37 (12)
Serum C-telopeptide (pM)	2,393 ± 1,456	2,647 ± 2,971	2,555 ± 1,736	2,608 ± 2,367

* Eleven participants had no evaluable spinal radiograph data.

† Data were collected through a calcium-intake questionnaire for 177 (placebo), 187 (100 IU), 207 (200 IU), and 203 (400 IU) participants.

Table 3. Summary of Vertebral Fracture Analyses for Entire Study Cohort, for Subgroup with One to Five Prevalent Vertebral Fractures, and for 3-Year Completers

| | | Treatment Groups | | |
| | | Nasal Spray Salmon Calcitonin | | |
	Placebo	100 IU	200 IU	400 IU
Entire study cohort	n = 270	n = 273	n = 287	n = 278
Participants with ≥1 new vertebral fractures [n (%)]	70 (26)	59 (22)	51 (18)	61 (22)
Relative risk (95% CI)		0.85 (0.60–1.21)	0.67 (0.47–0.97)*	0.84 (0.59–1.18)
Participants with ≥2 new vertebral fractures (n [%])	33 (12)	34 (13)	24 (8)	30 (11)
Odds ratio (95% CI)		1.02 (0.64–1.88)	0.65 (0.38–1.14)	0.87 (0.41–1.30)
New vertebral fractures/1,000 participant radiograph years	131	129	78*	111
Participants with 1–5 prevalent fractures	n = 203	n = 201	n = 207	n = 206
Participants with ≥1 new vertebral fractures [n (%)]	60 (30)	52 (26)	40 (19)	48 (23)
Relative risk (95% CI)		0.94 (0.65–1.36)	0.64 (0.43–0.96)*	0.78 (0.53–1.14)
Participants with ≥2 new vertebral fractures [n (%)]	30 (15)	32 (16)	18 (9)	23 (11)
Odds ratio (95% CI)		1.09 (0.64–1.88)	0.55 (0.30–1.02)	0.73 (0.41–1.30)
Three-year completers	n = 162	n = 152	n = 157	n = 155
Participants with ≥1 new vertebral fractures [n (%)]	59 (36)	49 (32)	40 (26)	42 (27)
Relative risk (95% CI)		0.91 (0.62–1.33)	0.66 (0.44–0.99)*	0.71 (0.48–1.05)

*200-IU versus placebo $P < 0.05$.
CI = confidence interval.

continued prematurely in the placebo group had a significantly higher percentage decrease in lumbar spine bone mineral density compared with those who discontinued in the active-treatment groups.

Vertebral Fractures

Follow-up radiographs were obtained for 1,108 of the participants (270 in the placebo group, 273 in the 100-IU group, 287 in the 200-IU group, and 278 in the 400-IU group). There was a 33% reduction in the relative risk of

Figure 1. Cumulative percentage of participants with at least one new fracture per year. Number of participants with follow-up radiographs (placebo = 270, 100 IU = 273, 200 IU = 287, 400 IU = 278). The asterisk indicates $P < 0.05$ versus placebo.

developing a new vertebral fracture in the salmon calcitonin nasal spray 200-IU group compared with placebo (RR = 0.67, 95% CI: 0.47 to −0.97, $P = 0.03$; Table 3 and Figure 1). The number of women with multiple new vertebral fractures (2 or more new vertebral fractures) was reduced by 35% ($P = 0.13$), and the number of new vertebral fractures per 1,000 participant radiograph years was reduced by 40% ($P = 0.02$) in the 200-IU group compared with the placebo group. Among women with one to five prevalent vertebral fractures at baseline, there was a 36% (RR = 0.64, 95% CI: 0.43– to 0.96, $P = 0.03$) reduction in the risk of developing a new vertebral fracture and a 45% ($P = 0.06$) reduction in the number with more than one new vertebral fracture (Table 3).

An analysis among participants who received the study drug for at least 3 years or who had a fracture during the first 3 years of treatment was performed to determine whether the high discontinuation rate had influenced the response to treatment. The results for the salmon calcitonin nasal spray 200-IU group were statistically and clinically significant and were similar to those observed in the main analysis (Table 3). In this "post hoc" analysis, based on the Kaplan-Meier survival curve, 11 women needed to be treated for 3 years with 200 IU salmon calcitonin nasal spray to prevent one vertebral fracture.

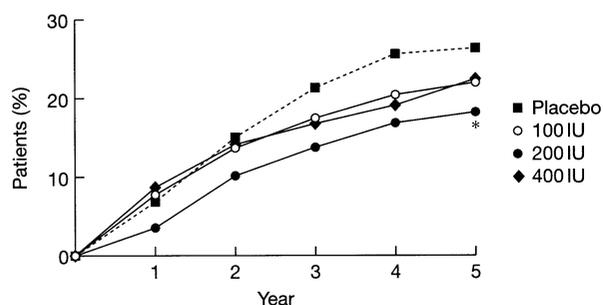There were no significant differences in any of these

Table 4. Summary of Nonvertebral Fracture Analyses

| | | Treatment Group | | |
| | | Nasal Spray Salmon Calcitonin | | |
	Placebo	100 IU	200 IU	400 IU
Number of participants at risk	(n = 305)	(n = 313)	(n = 315)	(n = 312)
All nonvertebral fractures				
Participants [n (%)]	48 (16)	32 (10)	46 (15)	41 (13)
Relative risk (95% CI)	—	0.64 (0.41–0.99)*	0.88 (0.59–1.32)	0.81 (0.53–1.23)
Hip or femoral fractures				
Participants [n (%)]	9 (3)	1 (0.3)	5 (2)	7 (2)
Relative risk (95% CI)	—	0.1 (0.01–0.9)*	0.5 (0.2–1.6)	0.8 (0.3–2.0)
Arm fractures†				
Participants [n (%)]	16 (5)	6 (2)	13 (4)	14 (5)
Relative risk (95% CI)	—	0.4 (0.1–0.9)*	0.75 (0.36–1.56)	0.84 (0.41–1.72)

*$P <0.05$ versus placebo.
† Fractures of the humerus, radius, ulna, or wrist.
CI = confidence interval.

outcomes when the 100-IU and 400-IU treatment groups were compared with the placebo group.

Nonvertebral Fractures

A total of 167 women had a nonvertebral fracture during the study (Table 4). Compared with placebo, the percentages of participants with nonvertebral fractures were significantly lower in the salmon calcitonin nasal spray 100-IU group ($P <0.05$), but not in the 200-IU or 400-IU groups.

The small number of hip and femoral fractures precluded a meaningful statistical analysis. There was a nonsignificant reduction in the risk of hip fracture in the 200-IU group (RR = 0.5, 95% CI: 0.2– to 1.6). There was a significant reduction in hip fractures in the 100-IU group (RR = 0.1, 95% CI: 0.01– to 0.9, $P = 0.04$), but not in the 400-IU group.

The number of fractures of the arm (humerus, radius, ulna, wrist) was also small (Table 4). There was a significant 64% reduction in the risk of fractures of the arm in participants receiving 100 IU salmon calcitonin nasal spray (RR = 0.36, 95% CI: 0.1– to 0.9, $P = 0.03$), but the reductions in risk in the 200-IU and 400-IU groups were not statistically significant.

Bone Mineral Density

Lumbar spine bone mineral density did not change substantially in the placebo group during the study. At year 1 and year 2, there was a significant increase ($P <0.05$) in lumbar spine bone mineral density in all calcitonin groups compared with placebo (Figure 2). At year 3, the increase in lumbar spine bone mineral density was statistically significantly different from placebo ($P = 0.01$) in only the 400-IU group. Lumbar spine bone mineral density in each salmon calcitonin nasal spray treatment group was increased significantly from baseline at each time point during the 5 years ($P <0.01$). No clinically

significant effect of treatment on bone mineral density was apparent at the femoral neck or trochanter. At the Ward's triangle, there was a 1.5% to 2.0% increase compared with placebo over 5 years in the salmon calcitonin nasal spray 200-IU group, which was statistically significant at years 1 ($P <0.01$) and 2 ($P <0.05$).

Markers of Bone Metabolism

Serum C-telopeptide levels decreased significantly from baseline in the 200-IU and 400-IU salmon calcitonin nasal spray groups at all time points up to year 5 ($P <0.05$; Figure 3). When the overall effect (average effect from baseline to year 5) was considered, the 200-IU and 400-IU doses produced statistically significant suppression compared with placebo (200 IU: −12%, $P = 0.01$; 400 IU: −14%, $P = 0.008$). Compared with placebo, serum bone-specific alkaline phosphatase levels decreased significantly in the 200-IU group at each time point ($P <0.05$)
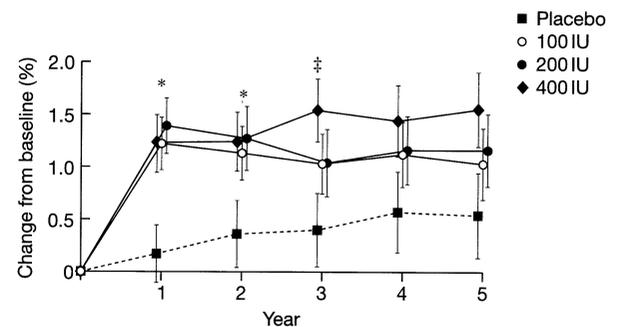

Figure 2. Lumbar spine bone mineral density, mean percentage change from baseline (± SEM). Number of participants with at least 1 postbaseline evaluation (placebo = 268, 100 IU = 273, 200 IU = 280, 400 IU = 274). A single asterisk indicates $P <0.05$ versus placebo; a double asterisk indicates $P <0.01$ versus placebo.
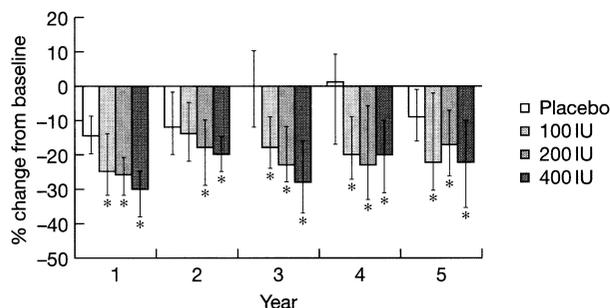
Figure 3. Serum C-telopeptide levels, median percentage change from baseline (± 95% confidence intervals). Number of participants with a least 1 postbaseline evaluation (placebo = 258, 100 IU = 262, 200 IU = 273, 400 IU = 262). A single asterisk indicates P < 0.05 change from baseline.

and in the 100-IU and 400-IU groups up to year 3 (*P* <0.01). There were statistically significant decreases in serum osteocalcin levels in the active treatment groups as compared with baseline, but no significant differences were observed compared with placebo. Antibodies that bind salmon calcitonin at titers greater than 1,000 developed in 74 participants (26%) in the 100-IU group, 94 (29%) in the 200-IU group, and 94 (34%) in the 400-IU group. The presence of high titers of antibodies did not influence the effect of salmon calcitonin on the risk reduction of new vertebral fractures.

Adverse Effects

The distribution of adverse effects was similar among the salmon calcitonin nasal spray and placebo groups, except for a significant increase in rhinitis related to the study drug (defined as nasal congestion, nasal discharge, or sneezing), which occurred in 22% of active-treated participants compared with 15% of placebo participants (*P* <0.01). Ninety-seven percent of nasal events in the calcitonin-treated groups and 91% of nasal events in the placebo group were of mild or moderate severity (calcitonin: 67% mild, 30% moderate; placebo: 64% mild, 27% moderate). Headache was reported less frequently in the salmon calcitonin nasal spray groups (4%) than in the placebo group (7%, *P* = 0.03).

DISCUSSION

The results of this 5-year clinical trial show that 200 IU of salmon calcitonin nasal spray per day significantly reduces the risk of new vertebral fractures by 33% to 36% in postmenopausal women with low bone mass or prevalent vertebral fractures. Among women with one to five vertebral fractures at baseline, 11 needed to be treated for 3 years to prevent a new vertebral fracture. The effect on vertebral fractures was accompanied by a modest increase in lumbar spine bone mineral density and a decrease in bone resorption.

Previous studies with parenteral or nasal salmon calcitonin, principally in participants with Paget's disease, have suggested that resistance may develop with continued use, potentially because of antibody formation, down-regulation of receptor sites, or counter-regulatory mechanisms (1,38–41). The results of this study, however, demonstrate a sustained effect in terms of reduction of fracture risk at the spine, maintenance of improved bone mineral density, and suppression of bone turnover during 5 years of observation.

Although vertebral fractures are the usual presenting manifestation of osteoporosis (42) and are associated with substantial morbidity (43), fractures of the hip have greater morbidity, mortality, and cost (43). Although definite conclusions on the risk of hip fracture cannot be drawn from our study, which was not designed to examine such effects, we did observe a nonsignificant 48% reduction in the risk of hip fracture in the salmon calcitonin nasal spray 200-IU group compared with the placebo group. The significant benefit observed in the 100-IU group may be the result of chance: the observed incidence (only one event, 0.3%) is about one-quarter of the incidence observed in active-treatment groups in other studies (44,45) as well as in the 200-IU group in this study. Further studies are indicated to determine the effect of salmon calcitonin nasal spray on the risk of hip fracture.

Salmon calcitonin nasal spray was well tolerated in these elderly women. The rate and reasons for discontinuation were distributed equally among treatment groups. Intolerance to the nasal spray did not contribute significantly to study discontinuation; less than 5% of participants discontinued for this reason.

Although there was a persistent benefit on spinal bone mineral density during the 5 years of the study in the 200-IU group, salmon calcitonin nasal spray reduced fracture risk without substantial effects on bone mineral density. Furthermore, only a modest effect was observed on serum C-telopeptide levels as a marker of bone resorption, although these levels were evaluated only at yearly intervals; the effects of salmon calcitonin nasal spray on bone resorption markers may occur within weeks to months (46). Although there was an association between reduced fracture risk and a 6% to 8% increase in bone mineral density and a 60% decrease in markers of bone resorption in women with vertebral fractures who were treated with alendronate (47–49), the results of this study and a study of raloxifene (45) show that approved osteoporosis therapies can reduce the risk of vertebral fractures without substantial improvement in bone mineral density or reduction in markers. How salmon calcitonin reduces the risk of fractures is not known; a decrease in bone turnover, particularly of the bone resorption component, may be as important a determinant of antifracture efficacy as an increase in bone mineral density (50–52). An improvement in bone mineralization (30,53)

may also matter. It is also possible that salmon calcitonin may improve bone quality and strength. These factors may act together to reduce the osteoclast activation frequency and trabecular erosion depth with a consequent reduction in trabecular perforations, microfractures, and subsequent macrofractures.

The high dropout rate in the study may have affected our results. The study was started in 1991 and was the first multicenter study to assess the effect of a new drug with vertebral fractures as the endpoint. The relatively high discontinuation rate should be considered in view of the 5-year treatment duration (approximately a 12% dropout rate per year). One reason for the dropout rate might have been that the investigators were not blinded to the bone mineral density results. When the trial was being planned, it was not considered ethical in a 5-year study to withhold the bone mineral density results from the investigator and the participant. The approval of two new osteoporosis treatments in the United States (salmon calcitonin nasal spray and alendronate) and the relatively modest increase in bone mineral density (which participants and investigators may have perceived as lack of efficacy) may have caused some participants to discontinue prematurely. However, the statistical methods that we used to analyze our results were intention-to-treat analyses that considered time to event. Analysis of the baseline characteristics of participants at risk for a new vertebral fracture at years 3 and 4 shows that the groups were still well matched at these times, suggesting that selection bias had not occurred. Finally, the estimate of the treatment effect could have been biased if poor responders had discontinued in the 200-IU group while continuing in the placebo group. However, participants who discontinued prematurely in the placebo group had a significantly higher percentage decrease in lumbar spine bone mineral density compared with those who discontinued in the active-treatment groups.

We did not observe a dose–response in the reductions in the risk of new vertebral fractures. Such a dose–response would have strengthened the conclusions of the study, but its absence does not invalidate the results showing statistically and clinically significant antifracture efficacy in the salmon calcitonin nasal spray 200-IU dose group. However, the lack of antifracture efficacy in the 400-IU group was unexpected, especially because we observed significant biologic effects of the 400-IU dose on lumbar spine bone mineral density and serum C-telopeptide levels. Why these effects did not lead to significant reductions in the rate of vertebral fractures is not clear.

In summary, the results of our study demonstrate that salmon calcitonin nasal spray at a dose of 200 IU is a safe and effective treatment for postmenopausal women with established spinal osteoporosis.

PROOF STUDY GROUP

Jeffrey Miller, Jane Sherman, (Tampa, FL); Douglas Kiel, Jeannette Szaro (Providence, RI); Suthin Songcharoen, Susan Williams (Jackson, MS); Robert Bing-You, Donna Balyko, Linda Nye (Scarborough, ME); Theodore Rooney, Joyce Brown (Des Moines, IA); Maria Greenwald (Rancho Mirage, CA); Louis Avioli, Jane Muckerman (St. Louis, MO); Nelson Watts, Sandy Huff, Shelia O'Neal (Atlanta, GA); Stuart Weiss, Ann Berenbaum, Kay Bovee (San Diego, CA); Joel Block, Claire Mangels (Chicago, IL); Stanley Cohen, Kay Bransom (Dallas, TX); Sherwyn L. Schwartz, Sherry Shade (San Antonio, TX); John L. Stock, Judy Overdorf (Worcester, MA); Christine Schneyer, Pnina Schwartz (Baltimore, MD); Charles Sharp, Laura Tonaka (Pasadena, CA); Stuart Silverman, Connie Kawai (Beverly Hills, CA); Michael J. Maricic, Karen Liebler (Tucson, AZ); David J. Baylink, Marcella Fischer-deTabuenca, Sally Farley (Loma Linda, CA); Gratten Woodson, Amanda Colbert (Decatur, GA); Michael McClung, Betsy Love, (Portland, OR); William Shergy, Edie Turner (Huntsville, AL); Fred Singer, (Santa Monica, CA); Susan Silverton (Philadelphia, PA); Daniel Weiss, Ruth DeCurtis (Bedford, OH); Steve Harris, Kay Bolla, (San Francisco, CA); Angelo Licata, Gloria DePietro (Cleveland, OH); Meryl LeBoff, Kara Campobasso (Boston, MA); Charles H. Chesnut III, Margaret Pitzel (Seattle, WA); Paul D. Miller, Carol Wasnok, Susan Piper, Marianne Bovee, (Lakewood, CO); Bruce Ettinger, Arline Van Gessell, (San Francisco, CA); Claude Arnaud, Elena Pierini, (San Francisco, CA); Munro Peacock, Carol Manning (Indianapolis, IN); Rebecca Jackson (Columbus, OH); Robert Lindsay, Lillian Woelfert (West Haverstraw, NY); Roy Altman, Jose Puerto (Miami, FL); Avedis K. Khachadurian, Shelley Greenhaus (New Brunswick, NJ); Michael Schiff, Lisa Cave (Denver, CO); Michael Bolognese, Carol Bolognese (Gaithersburg, MD); Cyrus Cooper, Jill Pearson, Brigitte Stow (Southampton, UK); David Doyle, Jane Fleming (Chingford, UK); R. W. Jubb, Sue Brailssord (Birmingham, UK); Caje Moniz, Cettina Mangion (London, UK); David Reid, Rita Smith (Aberdeen, UK).

Coordinating center for x-ray evaluation and bone mineral density: University of California, San Francisco, San Francisco, CA. Harry Genant, Bo Fan, Pat Keegan, Chun Wu, Tom Fuerst.

Coordinating center for biomarkers: Jerry L. Pettis Veterans Administration Hospital, Loma Linda, CA.

David Baylink, Christine Nyght, Jossye Trivino-Cook, Jean Gonzalez, Aaron Eden, Aurora Petrilla, Shannon Boss, Shan Wong.

REFERENCES

1. Azria M. *The Calcitonins: Physiology and Pharmacology*. New York: Karger, 1989.
2. Baron B, Vignery A. Behavior of osteoclasts during a rapid change in their number induced by high doses of parathyroid hormone or calcitonin in intact rats. *Metab Bone Dis Relat Res*. 1981;2:339–346.
3. Kallio DM, Garant PR, Minkin C. Ultrastructural effects of calcitonin on osteoclasts in tissue culture. *J Ultrastruct Res*. 1972;39:205–216.
4. Holtrop ME, Raisz LG, Simmons HA. The effects of parathyroid hormone, colchicine, and calcitonin on the ultrastructure and the activity of osteoclasts in organ culture. *J Cell Biol*. 1974;60:346–355.
5. Raisz LG. Bone metabolism and its hormonal regulation. *Triangle*. 1983;22:83–84.
6. Chambers TJ, Azria M. The effect of calcitonin on the osteoclast. *Triangle*. 1988;27:53–60.
7. Ellerington MC, Hillard TC, Whitcroft SI, et al. Intranasal salmon calcitonin for the prevention and treatment of postmenopausal osteoporosis. *Calcif Tissue Int*. 1996;59:6–11.
8. Kapetanos G, Symeonides PP, Dimitriou C, et al. A double blind study of intranasal calcitonin for established postmenopausal osteoporosis. *Acta Orthop Scand Suppl*. 1997;275:108–111.
9. Overgaard K, Riis BJ, Christiansen C, et al. Nasal calcitonin for treatment of established osteoporosis. *Clin Endocrinol (Oxf)*. 1989;30:435–442.
10. Overgaard K, Riis BJ, Christiansen C, Hansen MA. Effect of salcatonin given intranasally on early postmenopausal bone loss. *BMJ*. 1989;299:477–479.
11. Overgaard K, Hansen MA, Nielsen VA, et al. Discontinuous calcitonin treatment of established osteoporosis — effects of withdrawal of treatment. *Am J Med*. 1990;89:1–6.
12. Overgaard K, Christiansen C. Long-term treatment of established osteoporosis with intranasal calcitonin. *Calcif Tissue Int*. 1991;49:S60–S63.
13. Overgaard K, Hansen MA, Jensen SB, Christiansen C. Effect of salcatonin given intranasally on bone mass and fracture rates in established osteoporosis: a dose–response study. *BMJ*. 1992;305:556–561.
14. Overgaard K. Effect of intranasal salmon calcitonin therapy on bone mass and bone turnover in early postmenopausal women: a dose–response study. *Calcif Tissue Int*. 1994;55:82–86.
15. Reginster JY, Denis D, Albert A, et al. 1-Year controlled randomized trial of prevention of early postmenopausal bone loss by intranasal calcitonin. *Lancet*. 1987;2:1481–1483.
16. Reginster JY, Denis D, Deroisy R, et al. Long-term (3 years) prevention of trabecular postmenopausal bone loss with low-dose intermittent nasal salmon calcitonin. *J Bone Miner Res*. 1994;9:69–73.
17. Reginster JY, Meurmans L, Deroisy R, et al. A 5-year controlled randomized study of prevention of postmenopausal trabecular bone loss with nasal salmon calcitonin, and calcium. *Eur J Clin Invest*. 1994;24:565–569.
18. Reginster JY, Deroisy R, Lecart MP, et al. A double-blind, placebo-controlled, dose-finding trial of intermittent nasal salmon calcitonin for prevention of postmenopausal lumbar spine bone loss. *Am J Med*. 1995;98:452–458.
19. Thamsborg G, Jensen JE, Kollerup G, et al. Effect of nasal salmon calcitonin on bone remodeling and bone mass in postmenopausal osteoporosis. *Bone*. 1996;18:207–212.
20. Rico H, Revilla M, Hernandez ER, et al. Total and regional bone mineral content and fracture rate in postmenopausal osteoporosis treated with salmon calcitonin: a prospective study. *Calcif Tissue Int*. 1995;56:181–185.
21. Gruber HE, Ivey JL, Baylink DJ, et al. Long-term calcitonin therapy in postmenopausal osteoporosis. *Metabolism*. 1984;33:295–303.
22. Wallach S, Farley JR, Baylink DJ, Brenner-Gati L. Effects of calcitonin on bone quality and osteoblastic function. *Calcif Tissue Int*. 1993;52:335–339.
23. Mosekilde L, Danielsen CC, Gasser J. The effect on vertebral bone mass and strength of long term treatment with antiresorptive agents (estrogen and calcitonin), human parathyroid hormone-(1–38), and combination therapy, assessed in aged ovariectomized rats. *Endocrinology*. 1994;134:2126–2134.
24. Geusens P, Boonen S, Nijs J, et al. Effect of salmon calcitonin on femoral bone quality in adult ovariectomized ewes. *Calcif Tissue Int*. 1996;59:315–320.
25. Karachalios T, Lyritis GP, Giannarakos DG, et al. Calcitonin effects on rabbit bone. Bending tests on ulnar osteotomies. *Acta Orthop Scand*. 1992;63:615–618.
26. Burnell JM, Baylink DJ, Chesnut CH, Teubner EJ. The role of skeletal calcium deficiency in postmenopausal osteoporosis. *Calcif Tissue Int*. 1986;38:187–192.
27. Kanis JA, Johnell O, Gullberg B, et al. Evidence for efficacy of drugs affecting bone metabolism in preventing hip fracture. *BMJ*. 1992;305:1124–1128.
28. Wu CY, Li J, Jergas M, Genant HK. Comparison of semiquantitative and quantitative techniques for the assessment of prevalent and incident vertebral fractures. *Osteoporosis Int*. 1995;5:354–370.
29. Genant HK, Jergas M, Palermo L, et al. Comparison of semiquantitative visual and quantitative morphometric assessment of prevalent and incident vertebral fractures in osteoporosis. *J Bone Miner Res*. 1996;11:984–996.
30. Lu Y, Mathur AK, Blunt BA, et al. Dual x-ray absorptiometry quality control. comparison of visual examination and process-control charts. *J Bone Miner Res*. 1996;11:626–637.
31. Lu Y, Ye K, Mathur AK, et al. Comparative calibration without a gold standard. *Stat Med*. 1997;16:1889–1905.
32. Farley JR, Hall SL, Ilacas D, et al. Quantification of skeletal alkaline phosphatase in osteoporotic serum by wheat germ agglutinin precipitation, heat inactivation, and a two-site immunoradiometric assay. *Clin Chem*. 1994;40:1749–1756.
33. Garnero P, Grimaux M, Demiaux B, et al. Measurement of serum osteocalcin with a human-specific two-site immunoradiometric assay. *J Bone Miner Res*. 1992;7:1389–1398.
34. Gundberg CM. Biology, physiology, and clinical chemistry of osteocalcin. *J Clin Ligand Assay*. 1998;21:128–138.
35. Singer FR, Aldred FP, Neer RM, et al. An evaluation of antibodies and clinical resistance to salmon calcitonin. *J Clin Invest*. 1972;51:2331–2338.
36. Cox DR. Regression models and life-tables. *J Royal Stat Soc (Series B)*. 1972;34:187–220.
37. Littell R, Milliken G. SAS system for mixed models. Cary, North Carolina: SAS Institute, 1996:633.
38. Takahashi S, Goldring S, Katz M, et al. Downregulation of calcitonin receptor mRNA expression by calcitonin during human osteoclast-like cell differentiation. *J Clin Invest*. 1995;95:167–171.
39. Levy F, Muff R, Dotti-Sigrist S, et al. Formation of neutralizing antibodies during intranasal synthetic salmon calcitonin treatment of Paget's disease. *J Clin Endocrinol Metab*. 1988;67:541–545.

40. Muff R, Dambacher MA, Fischer JA. Formation of neutralizing antibodies during intranasal synthetic salmon calcitonin treatment of postmenopausal osteoporosis. *Osteoporosis Int.* 1991;1:72–75.

41. Grauer A, Raue F, Schneider HG, et al. In vitro detection of neutralizing antibodies after treatment of Paget's disease of bone with nasal salmon calcitonin. *J Bone Miner Res.* 1990;5:387–391.

42. Riggs BL, Melton LJ, III, O'Fallon WM. Drug therapy for vertebral fractures in osteoporosis: evidence that decreases in bone turnover and increases in bone mass both determine antifracture efficacy. *Bone.* 1996;18:197S–201S.

43. Cooper C. The crippling consequences of fractures and their impact on quality of life. *Am J Med.* 1997;103:12S–19S.

44. Black DM, Cummings SR, Karpf DB, et al. Randomized trial of effect of alendronate on risk of fracture in women with existing vertebral fractures. Fracture Intervention Trial Research Group. *Lancet.* 1996;348:1535–1541.

45. Ensrud K, Black D, Recker R, et al. The effect of 2 and 3 years of raloxifene on vertebral and non-vertebral fractures in postmenopausal women with osteoporosis. *Bone.* 1998;23(suppl 5): S174.

46. Kraenzlin ME, Seibel MJ, Trechsel U, et al. The effect of intranasal salmon calcitonin on postmenopausal bone turnover as assessed by biochemical markers: evidence of maximal effect after 8 weeks of continuous treatment. *Calcif Tissue Int.* 1996;58:216–220.

47. Chesnut CH III, McClung MR, Ensrud KE, et al. Alendronate treatment of the postmenopausal osteoporotic woman: effect of multiple dosages on bone mass and bone remodeling. *Am J Med.* 1995; 99:144–152.

48. Garnero P, Shih WJ, Gineyts E, et al. Comparison of new biochemical markers of bone turnover in late postmenopausal osteoporotic women in response to alendronate treatment. *J Clin Endocrinol Metab.* 1994;79:1693–1700.

49. Liberman UA, Weiss SR, Bröll J, et al. Effect of oral alendronate on bone mineral density and the incidence of fractures in postmenopausal osteoporosis. *N Engl J Med.* 1995;333:1437–1443.

50. Dempster DW. Exploiting and bypassing the bone remodeling cycle to optimize the treatment of osteoporosis. *J Bone Miner Res.* 1997;12:1152–1154.

51. Garnero P, Hausherr E, Chapuy MC, et al. Markers of bone resorption predict hip fracture in elderly women: the EPIDOS Prospective Study. *J Bone Miner Res.* 1996;11:1531–1538.

52. Cummings SR, Black DM, Vogt TM, for the FIT Research Group. Changes in BMD substantially underestimate the anti-fracture effects of alendronate, and other antiresorptive drugs. *J Bone Miner Res.* 1996;11:(suppl 1) S102:29.

53. Chavassieux PM, Arlot ME, Reda C, et al. Histomorphometric assessment of the long-term effects of alendronate on bone quality and remodeling in patients with osteoporosis. *J Clin Invest.* 1997; 100:1475–1480.