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Bioavailability and Biological Efficacy of a New Oral Formulation of Salmon Calcitonin in Healthy Volunteers*

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ABSTRACT

Salmon calcitonin (SCT) is a well-tolerated peptide drug with a wide therapeutic margin and is administered parenterally for long-term treatments of bone diseases. Its clinical usefulness would be enhanced by the development of an orally active formulation. In this randomized crossover double-blinded phase I trial, controlled by both a placebo and a parenteral verum, we have tested a new oral formulation of SCT associated with a caprylic acid derivative as carrier. Eight healthy volunteers received single doses of 400, 800, and 1200 μg of SCT orally, a placebo, and a 10-μg (50 IU) SCT intravenous infusion. SCT was reliably absorbed from the oral formulation, with an absolute bioavailability of 0.5–1.4%, depending on the dose. It induced a marked, dose-dependent drop in blood and urine C-terminal telopeptide of type I collagen (CTX), a sensitive and specific bone resorption marker, with the effects of 1200 μg exceeding those of 10 μg intravenously. It also decreased blood calcium and phosphate, and increased the circulating levels of parathyroid hormone (PTH) and, transiently, the urinary excretion of calcium. It was well-tolerated, with some subjects presenting mild and transient nausea, abdominal cramps, diarrheic stools, and headaches. This study shows that oral delivery of SCT is feasible with reproducible absorption and systemic biological efficacy. Such an oral formulation could facilitate the use of SCT in the treatment of osteoporosis and other bone diseases. (J Bone Miner Res 2002;17:1478–1485)

Key words: calcitonin, oral administration, intestinal absorption, bone resorption markers, healthy humans

INTRODUCTION

CALCITONIN IS used in the management of bone disorders such as osteoporosis, Paget's disease, and Südeck's algoneurodystrophia.[1–5] Although the excellent tolerability of this treatment was recognized early, its convenience was limited during some times by the requirement of daily injections, because the peptide is readily degraded and practically not absorbed through the gastrointestinal tract.[6–8] Subsequently, noninjectable formulations using the nasal and the rectal routes were developed for salmon calcitonin (SCT), taking advantage of its high affinity for the human receptor and slow elimination rate, which allows the delivery of smaller amounts than the human analog.[9–16] Nevertheless, the quest for an orally active formulation remained forceful.[17,18] Considering its administration over several months or years, its good safety profile, and its wide therapeutic margin, calcitonin has been regarded as a prototype candidate for the challenge of making a peptide drug bioavailable through the digestive tract.

Two main pathways have been explored for this pharmaceutical development.[17,19,20] Protection of SCT against

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proteolytic degradation in the intestinal lumen was attempted using acid additives, protease inhibitors, water/oil emulsions, slow-release and mucoadhesive microparticle formulations, polymer-based hydrogels, or direct conjugation to fatty acids or short polymers. On the other hand, amelioration of the mucosal penetration of SCT was sought using permeation enhancers such as bile acids derivatives, acylcarnitine, detergents, phospholipids, fatty acids, or oil emulsions. Various formulations associating SCT with such additives have been patented and tested in animals over the past years,[17,21–24] but to our knowledge, no results have been published to date regarding the biological efficacy of an oral form of SCT in humans, which represents the crucial outcome for this development. Two small phase I trials for enteric-coated preparations combining SCT with citric acid and either taurodeoxycholic acid or laurylcarnitine were reported briefly and showed an average bioavailability of 0.03% and 0.38%, respectively.[25] A lipid-based preparation showing encouraging effects in animals[22] was claimed to enter a phase III trial in osteoporosis patients, but the unsuccessful results were never reported in the scientific literature.[26]

A new family of low molecular weight carriers, derived from *N*-acylated amino acids, have been developed recently.[27] They are thought to increase selectively the mucosal uptake by inducing conformational changes in the peptide molecules.[28] While forming noncovalent bonds with the carrier, the molecules undergo partial unfolding and may both relax their shape and expose inner lipophilic residues, thus facilitating their transmembrane passage.[29] Unlike traditional surfactants and detergents, this class of absorption promoters has a certain specificity for peptides and polyaminoglycans and is practically devoid of toxic activity toward the intestinal epithelial cells. During preclinical studies, products of this class have been shown to promote the systemic absorption of orally administered SCT with an excellent tolerability.

Considering the obvious therapeutic interest of an oral formulation of SCT based on this technology, we have designed this phase I clinical trial to verify that it was absorbed to a significant extent and produced the biological and metabolic changes expected in healthy humans, to evaluate its absolute bioavailability and to confirm its short-term safety and tolerability. In particular, the study aimed to compare the antiresorptive activity profiles of oral and injected SCT by measuring the blood levels and urine excretion of the C-terminal telopeptide of type I collagen (CTX), a highly sensitive and specific biological marker of bone resorption.[30]

MATERIALS AND METHODS

Study subjects

Eight healthy male volunteers (age, 22–37 years; weight, 65–86 kg) were included in the study after giving their written informed consent. Their good health was assessed by a complete medical examination and standard blood and urine tests, including thyroid and parathyroid hormones (PTH) and 25-hydroxyvitamin D_3 determinations. They had to refrain from strenuous exercise and drug consumption over the whole study duration and from any alcohol, nicotine, caffeine, or calcium-rich food for 2 days before each study day. On study mornings, they had to remain fasted and to note the time of urine emission. They were admitted in the investigation unit and a catheter was inserted in an arm vein for repeated blood sampling. They received standardized meals and beverages from 2 to 24 h after the administration of the test drug (i.e., at 11 a.m., 1 p.m., and 6 p.m.). At the end of the last study period, the medical and laboratory examinations were repeated. The study protocol, information sheet, and consent form received approval from the Ethics Committee of the Faculty of Medicine in Lausanne on December 7, 2000.

Treatment protocol

The study followed a five-period randomized placebo-controlled crossover design. It was performed under double-blind conditions, except for the verum. It compared three single oral doses of SCT (400, 800, and 1200 μg) with an oral placebo and with a verum intravenous infusion of SCT (10 μg; i.e., 50 IU). The oral formulation consisted of tablets containing 400 μg of SCT (Novartis Pharma, Basle, Switzerland) and 225 mg of a caprylic acid derivative as specific carrier,[27] colyophilized from a sodium phosphate-buffered solution. The oral placebo consisted of matching lactose tablets. During the four oral treatment periods, the subjects received 0, 1, 2, or 3 tablets of the active compound and, respectively, 3, 2, 1, or 0 placebo tablets, according to a preestablished randomization list, with 300 ml of mineral water (Henniez, Henniez, Switzerland). The SCT infusion was prepared with commercial Miacalcic ampoules (50 IU/ml; Novartis Pharma) and administered over 1 h through a catheter in the arm opposite to blood sampling. The subjects had to remain fasting from the evening before until 2 h after drug administration.

Study measurements

Blood samples were drawn at predose time and 15, 30, and 45 minutes and 1, 1.5, 2, 3, 4, 6, 9, 12, and 24 h postdose. They were immediately centrifuged and the plasma was separated, frozen, and sent in one batch for determination of SCT concentrations by a sensitive and highly specific chemoluminescence-based sandwich immunoassay method, using mouse monoclonal and rabbit polyclonal antibodies. The assay had a quantification limit of 2.5 pg/ml, an intra-assay precision of 6.2%, and an interassay reproducibility of 16.6%, without cross-reactivity with human calcitonin. The following biological response markers were measured at predose time and 30 minutes and 1, 2, 3, 4, 6, 9, 12, and 24 h postdose: blood ionized calcium and pH, using a specific electrode device (ABL700; Radiometer, Copenhagen, Denmark); total serum calcium, phosphate, and albumin, using standard colorimetric methods (Synchron CX5 analyzer; Beckman Coulter, Fullerton, CA, USA); serum CTX, using a sandwich ELISA kit (Beta-Crosslaps; detection limit 0.01 ng/ml, intra- and interday variability $<5\%$; Roche Diagnostics, Meylan, France); and

parathyroid hormone (PTH), using a chemoluminescence immunoassay (detection limit 2 pg/ml, intra- and interday variability <7%; Nichols Institute Diagnostics, San Juan Capistrano, CA, USA). Serum 25-hydroxyvitamin D_3 and osteocalcin were determined in the predose and the 24-h postdose samples, using, respectively, a radioimmunoassay (RIA) kit (DiaSorin, Stilwater, MN, USA) and a sandwich immunoradiometric assay kit (Elsa-Osteo; CIS Bio/Schering, Gif-sur-Yvette, France). All the urines were saved in separate collections from morning rise to predose time, predose to 1.5 h, 1.5–3 h, 3–6 h, 6–9 h, 9–12 h, and 12–24 h postdose. Each collection was timed carefully and weighted for volume determination, and samples were kept frozen for determination of calcium, phosphate, creatinine, and CTX using an ELISA kit (Crosslaps; detection limit 50 ng/ml, intra- and interday variability <10%; Osteometer, Copenhagen, Denmark). All measurements were performed under blinded conditions.

Data analysis

The blood measurement data were averaged at each time according to treatment using geometric averages for plasma SCT, CTX, and PTH, considering their skewed distribution. Urinary excretion rates were calculated from the concentration, volume, and duration of each collection. The pharmacokinetics of SCT were assessed using standard noncompartmental calculations under Excel (version 2000; Microsoft, Redmond, WA, USA): data examination for the peak concentration C_{max} and time to peak t_{max}; log-linear regression for the terminal rate constant λ and resulting half-life $t_{1/2} = \text{Log}(2)/\lambda$; trapezoidal rule with extrapolation to infinity for the area under the curve (AUC); ratio of dose over AUC for the apparent systemic clearance CL/*F*; ratio of CL/*F* over rate constant for the apparent distribution volume *V*/*F*; and ratio of oral over intravenous AUCs divided by dose for the absolute bioavailability *F*.[31] In addition, a compartmental model was fitted to the plasma SCT data using the population pharmacokinetics software P-Pharm (version 1.5; Innaphase, Champs-sur-Marne, France). The efficacy data (CTX and PTH after log-transformation) were submitted to an ANOVA for repeated measurements accounting for the factors treatment, subject and time, and their respective interactions; the significance of the time-by-treatment interaction was assessed using the Huynh-Feldt correction for repeated measurements. Post hoc comparisons of means observed under active treatment versus placebo at corresponding times were performed using the Scheffé method under the protection of the global test for the time-by-treatment effect. Finally, the maximum percent decrease (PD_{max}) and the area under the effect curve (AUEC) were calculated from log-transformed CTX values reported to baseline and submitted to an ANOVA along with the pharmacokinetic parameters to test for a treatment effect. All statistical computations were performed with the Stata software (version 6; Stata Corp., College Station, TX, USA).

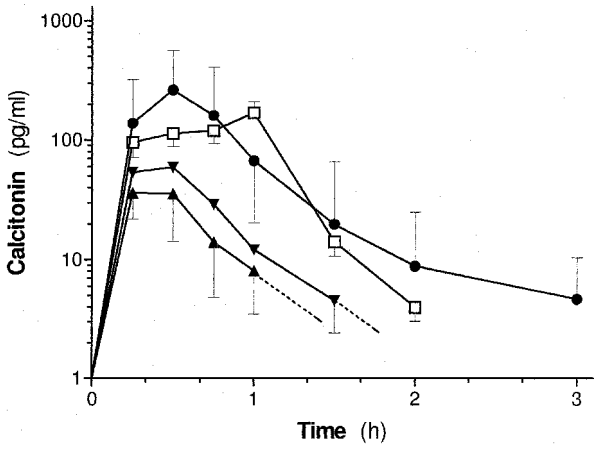

FIG. 1. Plasma SCT concentrations after three oral doses of SCT (▲, 400 μg; ▼, 800 μg; ●, 1200 μg) and after a 1-h intravenous SCT infusion (□, 10 μg; means ± SDs of log-transformed measurements in eight volunteers). The dotted lines indicate the average slope used to extrapolate AUC values to infinity at the lower doses.

RESULTS

Parmacokinetics

The plasma concentration profiles of SCT after the three oral doses and the intravenous infusion are depicted in Fig. 1. Concentrations were detectable up to 0.5–1.5 h after the dose of 400 μg, 1–2 h after 800 μg, and 1–4 h after 1200 μg given orally and were of the same order of magnitude than those observed after the 10-μg intravenous infusion. The pharmacokinetic parameters are summarized in Table 1. The data did not allow a precise characterization of the terminal rate constant and half-life. Therefore, these parameters were estimated only after the highest oral dose and after the infusion, and the AUCs were estimated after 400 μg and 800 μg were extrapolated to infinity based on the half-life calculated after 1200 μg. The average absolute bioavailability across all oral doses was 0.8%. It tended to increase with the dose, reaching 1.4% for 1200 μg ($p = 0.10$). The estimates of apparent clearance and distribution volume varied conversely. The peak SCT concentration corrected for dose and the time to peak followed a similar trend ($p = 0.13$ and $p < 0.0001$, respectively), suggesting some degree of nonlinearity in the absorption process. An additional population pharmacokinetic modeling showed that a one-compartment model with first-order absorption was appropriate to fit the data; the absorption rate constant was 2.4 ± 0.5 h^{-1}, resulting in an absorption half-life of 0.29 h; the oral bioavailability was 1.37 ± 1.02%; the absolute clearance was 64 ± 3 liters/h; and the distribution volume was 16.6 ± 0.8 liters; in accordance with an elimination half-life of 0.18 h. As indicated by this approach, the absorption would represent a rate-limiting step in the disposition of oral SCT.

Markers of bone metabolism

The serum levels and the urinary excretion rates of CTX are shown in Fig. 2. Both markers reflected a marked

TABLE 1. NON-COMPARTMENTAL PHARMACOKINETIC AND PHARMACODYNAMIC PARAMETERS OF ORAL AND INTRAVENOUS SALMON CALCITONIN (SCT).

Dose of SCT:	*400 μg oral*	*800 μg oral*	*1200 μg oral*	*10 μg intravenous*
Pharmacokinetic parameters:				
C_{max} (pg/mL)	60 ± 31	93 ± 66	370 ± 350	173 ± 37
t_{max} (h)	0.38 ± 0.13	0.44 ± 0.12	0.50 ± 0.13	0.97 ± 0.09
$t_{1/2}$ (h)	(not evaluable)		0.31 ± 0.09	0.28 ± 0.05
AUC (pg · h/mL)	31 ± 20	54 ± 41	273 ± 287	158 ± 31
CL/F (L/h)	18442 ± 11554	21947 ± 13406	7889 ± 5436	65 ± 12
V/F (L)	8191 ± 7019	9011 ± 4771	2994 ± 1657	26.2 ± 5.6
F (%)	0.54 ± 0.39	0.41 ± 0.24	1.44 ± 1.42	(reference 100%)
Pharmacodynamic parameters:				
PD_{max} (%)[a]	85.1 ± 9.1	89.5 ± 5.6	93.7 ± 2.8	93.3 ± 2.9
AUEC (Log · h)[b]	16 ± 6	17 ± 5	30 ± 11	23 ± 6

Means ± Standard Deviations in 8 Volunteers. C_{max}: Peak SCT Concentration, t_{max}: Time to Peak; $t_{1/2}$: Terminal Half-Life, AUC: Area Under the SCT Curve, CL/F: Apparent Systemic Clearance; V/F: Apparent Distribution Volume; F: Absolute Bioavailability, PD_{max}: Maximum Percent Decrease in Serum CTX, AUEC: Area Under the Curve of Log CTX, Reported to Baseline.

[a] An average value of 62 ± 10% was observed after placebo.

[b] An average value of 9 ± 2 Log · h was observed after placebo.

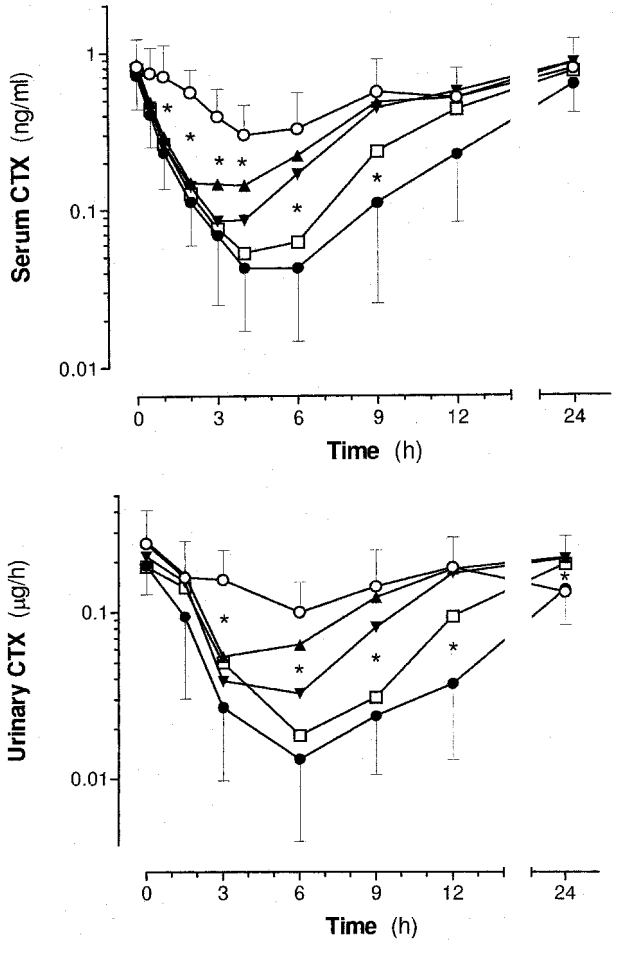

FIG. 2. Serum concentrations and urinary excretion rates of CTX after three oral doses of SCT (▲, 400 μg; ▼, 800 μg; and ●, 1200 μg), after a 1-h intravenous SCT infusion (□, 10 μg), and after a placebo (○, 153, means ± SDs of log-transformed measurements in eight volunteers). *Significant differences from placebo ($p < 0.05$).

dose-dependent inhibition of bone resorption after oral as well as intravenous SCT ($p < 0.0001$). The maximum percent decrease and the AUEC for serum CTX are summarized in Table 1. These efficacy results are in full agreement with the pharmacokinetic data; both the plasma SCT concentration and the effect profile of the 800-μg oral dose fell below those of the 10-μg intravenous infusion, and those of the 1200-μg oral dose reached higher levels, as shown in Fig. 3. Thus, considering both pharmacokinetic and pharmacodynamic criteria, the bioavailability of the oral SCT formulation can be roughly estimated around 1%. Although the pharmacokinetic profile of oral SCT displayed a somewhat higher variability compared with that of the intravenous administration, this had no significant influence on the variability of the response. Interestingly, both the 1200-μg oral dose and the 10-μg infusion of SCT decreased slightly the serum osteocalcin concentrations at 24 h (from 32 ± 12 ng/ml to 27 ± 9 ng/ml and 27 ± 8 ng/ml, respectively; $p = 0.004$).

Biochemical variables

The measurements of other variables also revealed dose-related effects of oral SCT. They confirmed the approximate equivalence of the 1200-μg oral dose and the 10-μg intravenous infusion. Oral SCT decreased to similar extents both the ionized blood calcium ($p = 0.015$; Fig. 4) and the total serum calcium ($p = 0.017$, not shown). All active doses exerted a transient calciuric effect ($p = 0.002$), evident on the first urine collection (0–1.5 h) and extending over the second collection (1.5–3 h) for the 1200-μg dose only; a slight decrease of calcium excretion was noticed from 9 to 12 h after this dose (Fig. 4). SCT decreased serum phosphate between 2 and 6 h compared with placebo ($p = 0.015$, not shown), without consistent effects on phosphaturia ($p = 0.2$). It did not affect blood pH ($p = 0.9$), albumin ($p = 0.2$), 25-hydroxyvitamin D ($p = 0.6$), or creatinine excretion rate

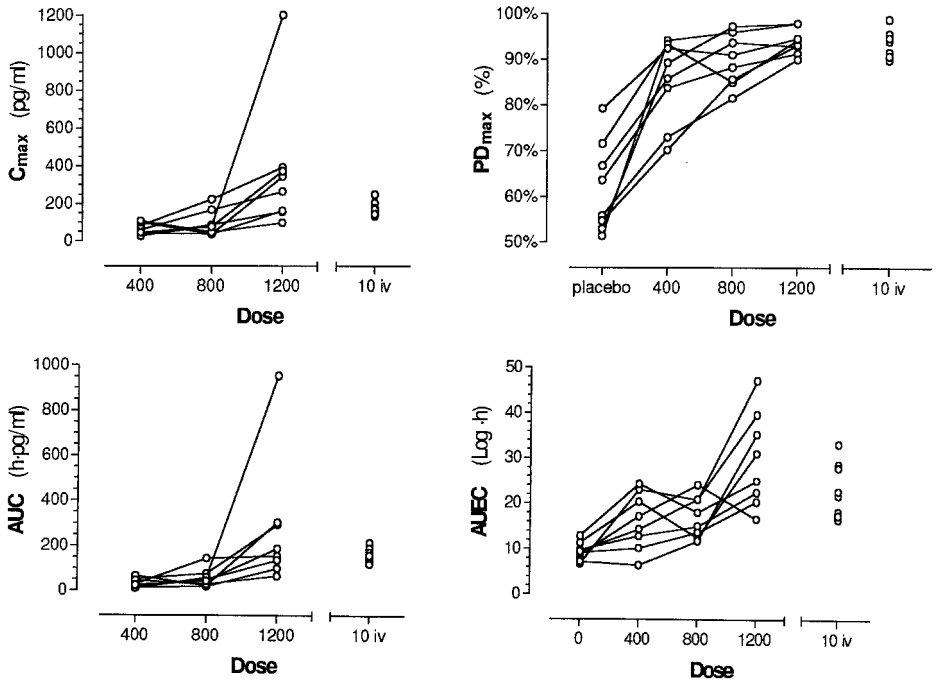

FIG. 3. Individual values of peak SCT concentrations (C_{max}), area under the SCT curve, peak percent decrease of serum CTX (PD_{max}), and AUEC after various oral doses of SCT (the 10-μg intravenous dose and the placebo).

($p = 0.6$). Finally, both the SCT infusion and the 1200-μg oral dose tended to produce a moderate <50% increase in serum PTH levels between 15 minutes and 12 h and 2 and 12 h, respectively ($p = 0.08$; Fig. 5).

Safety and tolerability

The study medication caused no serious problem. Altogether, 33 adverse events were recorded on 21/40 study periods, all of them being scored mild. The most frequent events were transient nausea and abdominal cramps, which occurred after 4/8 exposures to 1200 μg of oral SCT, 2/8 exposures to 10 μg of intravenous infusion, 1/8 exposure to 800 μg orally, and 1/8 exposure to 400 μg orally. Headaches were reported after 3/8 exposures to 10 μg intravenously, 2/8 exposures to 1200 μg orally, 2/8 exposures to 400 μg orally, and 1/8 exposure to 800 μg orally. Three subjects had one or two diarrheic stools after the oral intake of 1200 μg. Neither the measurements of vital signs nor the safety tests at study completion revealed any clinically significant abnormality.

DISCUSSION

Our results show that an oral formulation of SCT associated with a *N*-acylated amino acid derivative as carrier is able to produce all the biological effects of calcitonin in healthy volunteers. The intestinal absorption of the peptide remains limited, as indicated by both the pharmacokinetic and the pharmacodynamic criteria indicating a bioavailability of ~1%. Thus, ~1000 μg are required through the oral route to reproduce the concentrations and the effects of an intravenous infusion of 10 μg (50 IU). Consistent dose-concentration and dose-effect relationships can be observed, in line with a good reproducibility of the absorption despite this low bioavailability (Fig. 3). The pharmacokinetic parameters evaluated after the intravenous infusion are in fair agreement with values published earlier[32–36]; the shorter half-life and higher systemic clearance found here probably can be explained by progress in the determination methods, with increased specificity resulting in lower signal from cross-reactive metabolites. The lower terminal half-life values observed after oral administration, compared with those after intravenous infusion, suggest a slight slow-release effect of the oral formulation.

Among the study variables, serum CTX represented the most sensitive biological marker to reflect the rapid and profound inhibition of bone resorption induced by SCT. The higher variability observed in plasma SCT levels after oral administration compared with intravenous administration did not translate into a proportional variability in the CTX response. This probably is explained by SCT eliciting a near-maximal response at the applied dosage range, where the concentration-effect curve tends to flatten. On the other hand, serum CTX also displayed some decrease after placebo, probably related to food intake at breakfast and lunch.[37] Moreover, bone resorption is known to be affected by circadian rhythms, which may have influenced the response to both SCT and placebo over the follow-up period.[38] Urinary CTX excretion rate paralleled the blood levels, with comparable sensitivity and precision. The requirement of urine collections makes it difficult to repeat urinary measurement at shorter time intervals, this may introduce further imprecision (e.g., because of improper time or volume recording or incomplete bladder voiding). These results are in agreement with several other studies

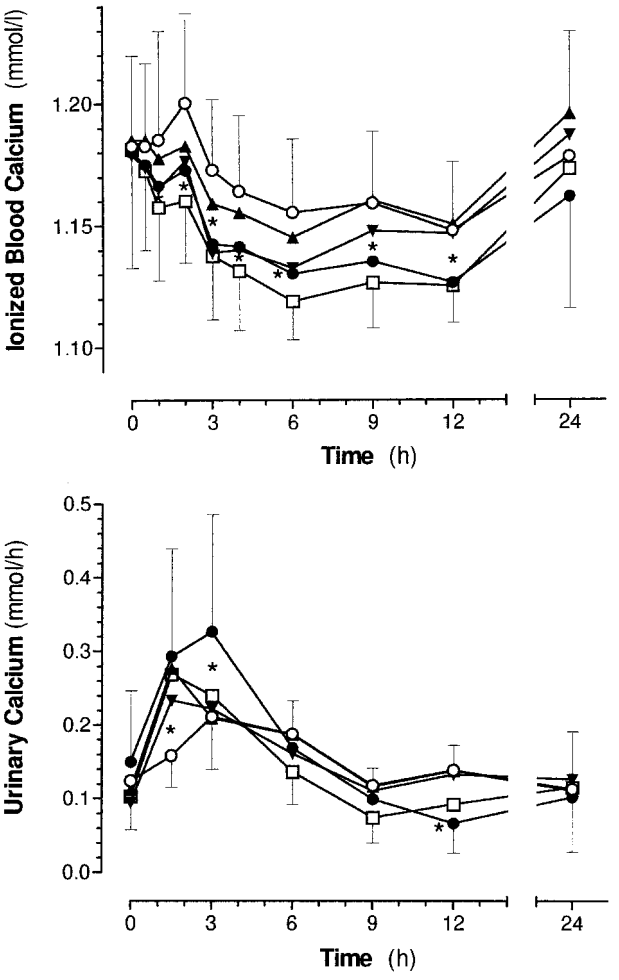

FIG. 4. Blood ionized calcium and urinary calcium excretion rate after three oral doses of SCT (▲, 400 μg; ▼, 800 μg; and ●, 1200 μg) after a 1-h intravenous SCT infusion (□, 10 μg) and after a placebo (○, means ± SDs of log-transformed measurements in eight volunteers). *Significant differences from placebo ($p < 0.05$).

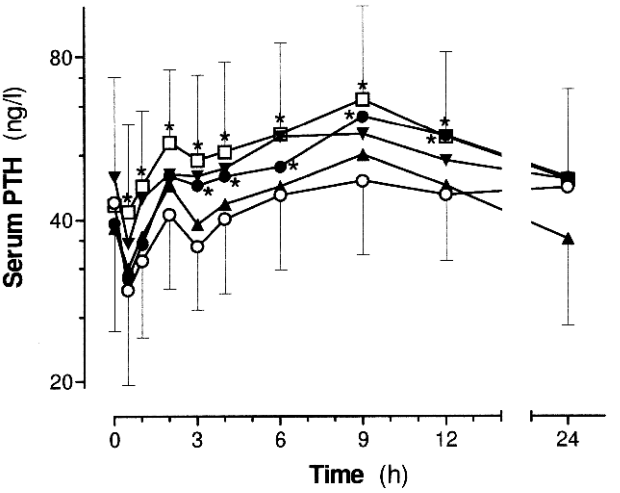

FIG. 5. Serum PTH concentrations after three oral doses of SCT (▲, 400 μg; ▼, 800 μg; and ●, 1200 μg) after a 1-h intravenous SCT infusion (□, 10 μg) and after a placebo (○, means ± SDs) of log-transformed measurements in eight volunteers. *Significant differences from placebo ($p < 0.05$).

having shown a marked effect of SCT on CTX or other bone resorption markers such as urinary pyridinoline, deoxypyridinoline, or hydroxyproline, either in healthy volunteers or in individuals with osteoporosis.[16,18,30,39–42] Our observation of a slight decrease in serum osteocalcin after the highest SCT doses is in line with the concept of coupling between bone resorption and formation. Although a decline of this formation marker has been reported regularly in osteoporosis or Paget patients after several weeks of calcitonin treatment, this trend appears detectable in healthy subjects already after a single administration of SCT.[3]

The performance of other metabolic markers such as calcemia, calciuria, phosphatemia, and serum PTH to assess the biological activity of SCT is clearly inferior. However, these measurements still have their importance to confirm that the administered preparation exerts the whole array of known biological effects of calcitonin.[1,16,43–46] In fact, had it relied only on CTX, this study could not have excluded a singular effect of the absorption enhancer on this marker, because the placebo did not contain the enhancer but merely lactose. Finally, the concomitant use of two control treatments, one placebo and one verum (the intravenous SCT infusion), increases the strength of the evidence of biological effectiveness of the oral SCT tested.

Thus, the challenge of making a peptide such as SCT absorbable through the oral route appears to have been taken up successfully. The formulation evaluated here seems to have satisfactory biopharmaceutical characteristics, without relying on sophisticated and costly manufacturing techniques. The tolerability of the preparation was good; it mainly reproduced the mild, transient effects, which were described after injectable nasal or rectal calcitonin and which were observed after the intravenous SCT infusion. The observation of isolated diarrheic stools after the highest oral dose, and not after the infusion, may suggest a direct secretory effect of high local SCT concentration on the intestinal mucosa, as described in animal experiments, or less probably another mechanism related to the presence of the carrier.

SCT as a single drug has an established, although limited, effectiveness in osteoporosis.[3,11,47,48] Peptides like SCT and PTH analog probably will keep an important place in the treatment of this condition.[49] Unlike other hormones, neurotransmitters, or cytokines, the peptidic agents used in the treatment of bone and mineral disorders have fairly wide therapeutic margins and satisfactory safety profiles and are applied over prolonged periods. Thus, the perspective of administering such drugs through the oral route with reliable absorption characteristics is highly attractive.

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