The Bioavailability of Intranasal Salmon Calcitonin in Healthy Volunteers with and Without a Permeation Enhancer

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Serum levels of radioimmunoactive salmon calcitonin (sCT) were determined in 10 healthy volunteers after intranasal administration (IN) of 100-, 205-, and 450-IU of sCT with 0.5% sodium tauro-24,25-dihydrofusidate (STDHF), a 200-IU commercial IN formulation, and a 100-IU intramuscular (IM) formulation. Relative to the IM dose, the bioavailabilities of the IN formulations containing 0.5% STDHF were 3.9, 7.9, and 7.4%, respectively. The 200-IU commercial formulation resulted in serum levels above the limit of detection in only 5 of 10 patients, with an average bioavailability of 1.6%.

KEY WORDS: calcitonin; nasal delivery; peptide delivery; osteoporosis.

INTRODUCTION

Calcitonin is a peptide hormone which influences calcium metabolism primarily via its action on the gut, kidney, and bone (1). Calcitonin has been used therapeutically for the treatment of hypercalcemia (2), Paget's disease (3), and more recently osteoporosis (4). Due to the chronic nature of the latter two diseases and the adverse reactions associated with parenteral calcitonin therapy (5), alternative, noninvasive routes of administration are being actively pursued. Recently, several intranasal (IN) formulations have become available commercially and have been used widely in Europe.

In general, the permeability of the nasal mucosa to peptides falls off sharply with increasing molecular weight (6,7). For peptides with more than 10 amino acids, bioavailabilities are in the 1–3% range. Despite its 32-amino acid size, the "biodisposability" of intranasal salmon calcitonin (sCT) has been reported to be 40% relative to intramuscular (IM) administration (8). This number is supported by clinical data which show that intranasal doses of sCT at two to three times the IM dose are effective in halting the progression of Paget's disease (9) and the loss of bone density in postmenopausal women (10). Other pharmacokinetic studies suggest that the bioavailability of IN sCT is much lower than the 40% bioactivity reported above (11,12). This discrepancy could be attributed to differences in formulation, differences in the sCT assay sensitivity, or the use of inappropriate physiological parameters to measure bioavailability. Alternatively, the pulsatile pharmacokinetic profile normally observed after IN administration may result in an enhanced biological response relative to parenteral administration. As part of our evaluation of nasal permeation enhancers, we have compared the bioavailability of the commercially available sCT formulation, Calcitonin-Sandoz Nasal Spray 100, to an sCT formulation containing 0.5% sodium tauro-24,25-dihydrofusidate (STDHF) as a permeation enhancer. Using a sensitive and specific radioimmunoassay, we have been able to measure a dose response after IN administration of 100-, 205-, and 450-U doses of sCT containing STDHF and have compared this to a 200-U dose of Calcitonin-Sandoz and a 100-U IM dose.

METHODOLOGY

Materials

sCT (sp act, ~5000 IU/mg) was purchased from Bachem Bioscience, Inc. (Philadelphia, PA) and STDHF was obtained from Leo Pharmaceuticals (Ballerup, Denmark). The Calcitonin-Sandoz and Calsynar (IM; Armour) formulations were obtained from commercial suppliers. Salmon calcitonin radioimmunoassay (RIA) kits,. RIK-6003, were obtained from Peninsula Laboratories (Belmont, CA). Human serum was purchased from Biocell Laboratories (Carson, CA). A single metered-dose nasal spray unit (Pfeiffer, Princeton, NJ) was used to administer the sCT-STDHF nasal formulation. sCT-STDHF formulations were prepared by reconstitution of solid sCT with a sterile isotonic (NaCl) solution of 0.5% STDHF in 20 m*M* acetate buffer, pH 5.0, and used immediately.

Protocol

This study was carried out at Charterhouse Clinical Research Unit, Ltd. (London), in 10 normal, healthy, nonpregnant subjects (7 males, 3 females) between 20 and 37 years of age. The subjects weighed between 60 and 80 kg and were all nonsmokers. Subjects passed a complete standard physical and upper respiratory examination. Subjects were excluded from the investigation if a history of alcohol or drug abuse existed, if they were receiving chronic intranasal medication, or if any respiratory abnormalities, infections, or allergies were noted. Informed consent was obtained from all subjects.

Subjects were dosed once on each of 5 consecutive days. Four doses were administered IN on days 1 through 4 and one dose was administered IM on day 5. The IN doses consisted of Calcitonin-Sandoz (200 U/dose) and three formulations (100-, 205-, and 450-U/dose) containing 0.5% STDHF. The nasal calcitonin doses were administered as one 100-μL spray per nostril using either the multidose spray unit supplied with the Calcitonin-Sandoz or two single-dose spray units for the sCT-STDHF formulations.

Blood samples were taken at 15 min pre- and 0, 5, 10, 15, 20, 30, 45, 60, 90, 120, 180, 240, and 300 min postadministration and placed immediately on ice (or refrigerated). Serum was obtained by centrifugation of the whole blood after

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incubation for 3 hr at 4°C. Serum was subsequently frozen at −20°C prior to analysis. sCT stability during blood sample preparation has been previously assessed by RIA following *in vitro* incubation of sCT in plasma for up to 24 hours (13).

RIA

All serum samples were assayed with a commercial RIA kit developed at Peninsula Laboratories. Rabbit anti-sCT antibodies, which were used as the primary antibodies, have demonstrated less than 3% (w/w) cross-reactivity with human calcitonin. All sCT standard curves were prepared using normal human serum as the standard diluent and each concentration was done in quadruplet. Experimental serum samples were not diluted prior to analysis and each sample was analyzed in duplicate. The sigmoidal standard curve of B/B_0 vs log[sCT], typical of competitive binding assays, was linearized using the logit transformation of B/B_0. The best-fit line was determined using a nonweighted, least-squares regression analysis. The coefficient of variation for the slopes was 13%. The radioimmunoassay quantitation range in human serum was 10 to 1280 pg/mL. The square of the coefficient of determination (r^2) in this range for the logit B/B_0 versus log[sCT] plots was greater than 0.99 for all assays.

Analysis of Data

All samples which showed sCT serum concentrations below the limit of quantitation (10 pg/mL) were set to 0 pg/mL. In a small number of subjects, randomly distributed among the dose groups, serum concentrations greater than 10 pg/mL prior to drug administration ($T = -15$ min) were measured and these values subtracted from the serum concentration at each subsequent time point. Estimations of the area under the curve of [sCT] versus time were calculated using the trapezoidal rule. The intranasal bioavailabilities were calculated relative to the intramuscular administration, adjusting for the dose.

$$\%F = \frac{AUC_{IN}}{AUC_{IM}} \times \frac{\text{dose } (\mu g) \text{ IM}}{\text{dose } (\mu g) \text{ IN}} \times 100$$

Since the RIA detects sCT mass, and not activity, bioavailabilities (*%F*) were based on the mass of sCT delivered, and not the specific activities. Concentrations (μg/mL) of sCT in the Calcitonin-Sandoz and the sCT-STDHF formulations were determined by a reverse-phase HPLC method (ODS column, mobile phase: 0.1% TFA, 33–60% ACN in 25 min) using the bulk sCT as a standard. The sCT concentration (μg/mL) for Calsynar was calculated based on an activity of 4000 IU/mg. IN bioavailabilities were calculated relative to the individual subject's IM and IN AUC values (intra *%F*) or as a ratio of the mean IN and IM AUC values (inter *%F*) at each dose level.

RESULTS

The average serum sCT concentration-versus-time profile for the 100-U IM dose (25 μg) is shown in Fig. 1. The sCT serum profile for IN administration of Calcitonin-Sandoz (40-μg dose) is shown in the inset to Fig. 2. Only 5 of 10 subjects showed blood levels above the limit of quantitation

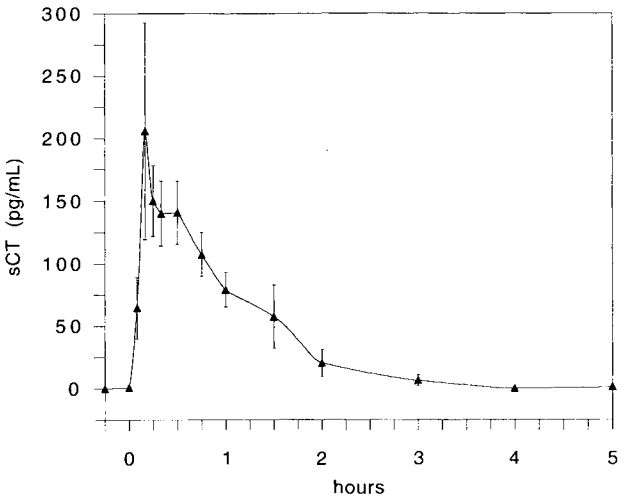

Fig. 1. Serum sCT concentrations (pg/mL) versus time after IM administration of 100 IU of sCT ($N = 10$). Error bars are SE.

(10 pg/mL). The individual subject profiles show a high degree of variability in the time to reach C_{max}, due in part to the closeness of the serum sCT levels to the assay detection limit. The rise in concentration seen at 4 and 5 hr is a result of single data points from subjects 1, 3, and 5.

The average serum profiles following IN sCT administration in formulations containing 0.5% STDHF are shown in Fig. 2. The 100-U dose (20 μg) resulted in serum concentration levels above 10 pg/mL in only 3 of the 10 subjects. The mean AUC, coefficient of variation (CV), C_{max}, T_{max}, and *%F* for all doses are summarized in Table I. All but two subjects had increasing dose responses to the three IN formulations containing STDHF.

The AUCs for the three IN doses containing STDHF were compared with the AUC values for the Calcitonin-Sandoz 200-U dose and the IM 100-U dose using a paired *t*

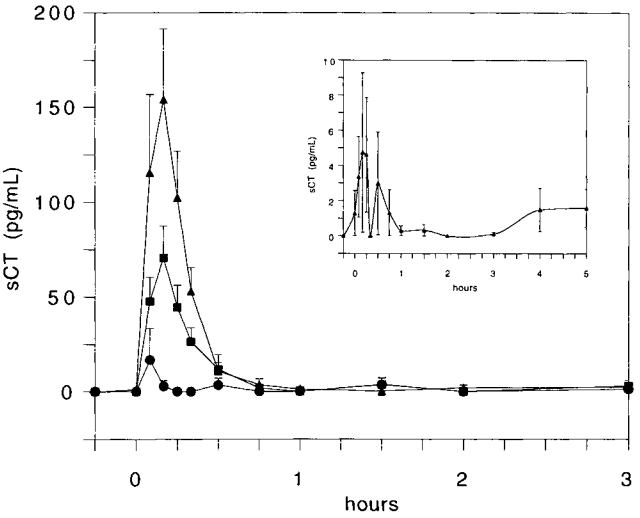

Fig. 2. Serum sCT concentrations (pg/mL) versus time after IN administration of 100 (●), 205 (■), and 450 (▲) IU of sCT with 0.5% STDHF ($N = 10$). Error bars are SE. Inset: Serum sCT concentrations (pg/mL) versus time after IN administration of 200-IU of sCT ($N = 10$). Error bars are SE.

Table I. Pharmacokinetic Parameters for IM and IN Administration of sCT[a]

Dose (μg)	AUC (pg min/mL)	CV (%)	C_{max} (pg/mL)	T_{max} (min)	%F inter	%F intra
IM, 100 U (25)	11,376	47	206	10	—	—
IN, 200 U (40)	294	148	4.8	10	1.6	2.9
IN, 100 U (20)[b]	355	165	16.8	5	3.9	3.1
IN, 205 U (41)[b]	1,477	68	70.7	10	7.9	9.6
IN, 450 U (90)[b]	3,041	76	154	10	7.4	10.3

[a] All dose volumes were 2 × 100 μL.
[b] IN formulations contain 0.5% STDHF.

test. The 205- and 450-U doses containing STDHF had significantly greater AUC values than the Calcitonin-Sandoz 200-U dose ($p = 0.05$), whereas the AUCs of the 100-U dose containing STDHF and the Calcitonin-Sandoz 200-U dose ($p = 0.05$) were not significantly different. The AUC for the IM dose was significantly greater than those for the 100-U IN dose and both 200-U IN doses but was not significantly greater than the 450-U dose containing STDHF ($p = 0.05$).

DISCUSSION

Detectable serum levels of sCT were observed for each intranasal formulation. All serum levels-versus-time curves after intranasal administration are characterized by a rapid absorption phase, with a 5- to 10-min T_{max}, followed by an almost equally rapid disposition. In contrast, the disposition of the IM dose is much slower, indicative of a slower rate limiting absorption into the blood from the injection site (Fig. 1).

For those formulations containing STDHF, the CV for the IN AUC at the 205- and 450-U doses are comparable to the IM dose (68 and 76 vs 47%), however, the 100-U dose shows a much greater CV (165%). The threefold increase in bioavailability at the two higher doses relative to the 100-U dose (10 vs 3.1%) may reflect the presence of saturable protease activity, nonspecific binding in the nasal mucosa, or the increasing thermodynamic activity at higher concentrations of sCT. The latter effect may be discounted based on preclinical work with human growth hormone and 0.5% STDHF, which showed a linear dose response over a fivefold concentration range (14). The active proteolytic environment of the nasal mucosa has been well documented (15), and the nonlinearity of the sCT dose response at low doses after IN administration also has been observed in a rat model (16). Recovery experiments in rats also have demonstrated that the enhancer effect of STDHF is transient, with the mucosal permeability returning to baseline values after 60–120 min, thus arguing against any permanent alternations in the mucosal permeability over the course of this trial (17).

The bioavailability of the 205-U dose with STDHF is 3.3 (intra) to 4.7 (inter) times higher than that of the commercial 200-U formulation. This difference can be accounted for by the effect of STDHF on nasal mucosa permeability. The commercial formulation dosed at 200 U is a simple aqueous solution containing benzalkonium chloride as a preservative. Although it has been suggested that benzalkonium chloride can act as a permeation enhancer (18), the effect in this study appears negligible. Interestingly, the 7–11% bioavailabilities obtained with the sCT formulations containing STDHF (205 and 450 IU) are very similar to an earlier clinical study with insulin (MW ~6000 Da) formulated with 1% STDHF (19). In another study carried out in growth hormone-deficient subjects, the intranasal bioavailabilities of human growth hormone (MW ~22,000 Da) formulations with 1% STDHF were in the 2–5% range (20), suggesting that the molecular volume of the protein may play a role in the STDHF-enhanced transport of proteins across the nasal mucosa. However, since direct comparisons of IN bioavailability in the presence of permeation enhancers must take into account baseline permeability, protease activity, nonspecific binding, and formulation, differences in bioavailability are not clear evidence of an exclusionary effect over this molecular weight range.

The blood levels of sCT obtained after intranasal administration of the 200-IU commercial formulation are lower than selected reports in the literature. Thamsborg *et al.* (21), using a RIA with a sensitivity limit of 3 pg/mL, report an AUC value of ~1100 pg min/mL for a 200-IU intranasal dose compared to the AUC value of 294 pg min/mL reported here. Their higher value results from an AUC calculation value using only three points (0, 15, and 240 min), thus minimizing the rapid clearance of sCT. In another publication comparing intrarectal and IN administered sCT administration in normal subjects, Buclin *et al.* (22) report an average AUC value for two sequential 200-IU doses of IN sCT of 4950 pg min/mL. Compared to the IM AUC values in this study, the %F would be 22%. The large IN AUC values in that study result from detectable plasma levels of sCT at 7 hr postdosing. This is an unusually extended profile for an IN dose of a hydrophilic peptide. Clearance of the mucosal surface by ciliary motion is rapid (10–15 min) and the high blood flow to the nasal mucosa makes a slow release of sCT unlikely. Alternatively, the points detected at longer times could represent immunoactive metabolic fragments of sCT, resulting in an overestimate of the true AUC value.

In spite of the low blood levels of sCT observed after IN administration with the commercial formulation, there is a large and growing body of clinical data supporting its efficacy in the treatment of Paget's disease and postmenopausal osteoporosis. Clinically, the IN dose administered is only twice that given IM (200- vs 100-IU). Since the bioavailability of the commercial IN dose in this study is only 1/50 of the IM dose, it can be concluded that the IM dose is given in large excess of that required for efficacy or that the pharmacokinetic profile of the IN dose results in a clear therapeutic advantage. A characteristic of the IN profile is a large C_{max}/AUC ratio. Although the %F for the 205- and 450-IU formu-

lations with STDHF are in the 7–8% range, the *%F* based on C_{max} values are approximately 20%.

The enhanced absorption of sCT in the presence of 0.5% STDHF was accompanied by a limited transient irritation of the nasal mucosa in some subjects. Irritation in those subjects diminished with repeated dosing. Mild facial flushing was also noted in patients who received the enhanced formulations at 205- and 450-IU. Facial flushing is a side effect of the parenteral administration of sCT and, as reported previously (23), was not observed for the commercial IN formulation, consistent with the low blood levels observed.

In conclusion, it is possible to enhance the absorption of sCT across the nasal mucosa in a dose-dependent manner using STDHF as a permeation enhancer. The increased bioavailability, the high C_{max}, and the acceptable CV values observed for these formulations could lead to reduced clinical doses of sCT and a convenient noninvasive delivery system for this important therapeutic hormone. Further clinical studies designed to examine the effects of lower STDHF concentrations on bioavailability and nasal irritation are currently in progress.

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