Buccal Transmucosal Delivery of Calcitonin in Rabbits Using Thin-Film Composites

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Purpose. Salmon Calcitonin (sCT) is used to treat hypercalcemia resulting from Paget's disease and osteoporosis. sCT is available either in a sterile injectable form or nasal spray. Alternative and more cost-effective dosage forms for the delivery of calcitonin are needed. We sought to deliver sCT transmucosally using a previously reported mucoadhesive bilayer thin-film composite (TFC) via the buccal route.
Methods. Forty micrograms of salmon calcitonin (200-IU) was loaded on preformed TFCs. *In vitro* release of sCT from TFCs was monitored in phosphate-buffered saline (10 mM, pH 7.4) at 37°C. Female New Zealand White rabbits (n = 6) were dosed with 40 μg of sCT either by injection via the ear vein or by applying sCT-loaded TFCs directly on the buccal pouch. Blood was collected at various times, and the plasma sCT and calcium concentrations were quantified. WinNonlin® was used to determine the relevant pharmacokinetic parameters.
Results. *In vitro*, over 80% of sCT was released from the TFCs within 240 min. Super Case-II transport was indicated as the primary release mechanism. Rabbits injected intravenously had C_{max}, Cls, Vss, and $AUC_{0\text{-}inf}$ values of 75.1 ± 6.5 ng/mL, 20.7 ± 3.3 mL/min, 637 ± 141 mL, and 1925 ± 237 ng*min/mL, respectively. Rabbits dosed via the buccal route had C_{max}, Cls, and $AUC_{0\text{-}400\ min}$ values of 4.6 ± 1.6 ng/mL, 22.0 ± 5.9 mL/min, and 842.9 ± 209.7 ng*min/mL, respectively. The relative bioavailability for rabbits treated with the TFCs was 43.8 ± 10.9% with a CV of 24.9%. The reductions in plasma calcium levels after administration of sCT by both the intravenous and buccal route were comparable.
Conclusions. The TFCs effectively delivered therapeutically efficacious amounts of sCT across the buccal mucosa in rabbits.

KEY WORDS: Eudragit; Polycarbophil; calcium; wax; film; salmon calcitonin; mucoadhesive.

INTRODUCTION

Osteoporosis is the most common metabolic bone disease in older people. A reduction in bone mass may lead to fractures, especially of the vertebrae, hips, and wrists. In the United States today, 10 million people have osteoporosis and 18 million more have low bone mass, placing them at increased risk for the disease (1). Calcitonin (CT) is a 32-amino acid peptide that has a molecular weight of 3432 Da. CT is secreted by the parafollicular cells of the thyroid glands in mammals and by the ultimobronchial glands in birds and fish (2). The basic structure of CT is characterized by a disulfide bridge between the two cysteine residues at positions 1 and 7 and the C-terminal proline amide moiety (3). CT has been used therapeutically for treatment of hypercalcemia, Paget's disease, and osteoporosis (4). It functions primarily by inhibiting bone resorption and by reducing the renal tubular reabsorption of calcium in the kidneys (2,5). Four CTs (porcine, human, eel, and salmon) are available for clinical use in humans. However, salmon calcitonin (sCT) has been extensively used because it is more effective than the other CTs (6).

Like many other peptide-based therapeutics, poor absorption and rapid proteolytic degradation have hindered the development of orally administered CT products. Currently, CT is available either as a relatively expensive sterile injectable or as a sterile nasal spray (7). Typical therapy with sCT requires daily dosing for an extended period of time. The current cost of calcitonin therapy for the treatment of osteoporosis is roughly $4,872 per year for daily injections of 100 IU per 0.5-mL injection (7). Moreover, chronic parenteral administration of CT, which may cause adverse side effects, is inconvenient for patients. Nasal sprays may have increased patient compliance over parenteral injectables but can cause nasal side effects, such as sneezing, itchy nose, rhinitis, nasal congestion, etc. (8). In addition, the reported bioavailability of sCT by the nasal route in humans is only 1.6% (9). Thus, alternative, more cost-effective, and less-invasive routes of administration of sCT that do not require a sterile dosage form are needed. Various researchers have investigated different dosage forms to deliver sCT by different routes. Besides the parenteral and nasal routes mentioned above, sCT has been delivered by several other routes (10–18).

The oral buccal route has been explored to administer drugs either locally or systemically. The buccal mucosa, alone with other mucosal tissues, has been investigated as a potential site for the delivery of macromolecular therapeutics such as peptides and proteins (19). The buccal mucosa is accessible and has lower enzymatic activity compared with the gastrointestinal tract. Also, buccal mucosa is potentially more tolerant to permeation enhancers in comparison with nasal mucosa (19). The delivery of drugs via the buccal mucosa offers a number of advantages over oral delivery, especially for those drugs that have poor solubility, poor oral bioavailability, and/or those drugs that suffer from extensive first-pass metabolism in the liver. Conceivably, buccal delivery systems may provide for easy administration with little or no irritation, thereby increasing patient compliance. Moreover, the efficacy of delivering sCT via the buccal mucosa has been established (12–14). Nakada *et al.* (12) studied the buccal delivery of calcitonin in rats as early as 1988. They observed a 1.6% decrease in plasma calcium levels. This decrease could be further augmented by co-administration of absorption enhancers, such as sodium deoxycholate, sodium taurocholate, sodium laurylsulphate, and dihydroxy bile salts (12). Heiber *et al.* (13) studied the absorption of sCT from both solutions and tablets in mongrel dogs and demonstrated that therapeutic levels of sCT can be achieved by the buccal route. Alur *et al.* (14) reported an apparent bioavailability of as high as 37% when sCT was administered via buccal tablets, which contained a natural oligosaccharide gum as a sustained-release and mucoadhesive component.

We have developed a novel bioerodable mucoadhesive bilayer thin-film composite (TFC) that strongly adheres to the buccal tissue for up to 4 h and allows for unidirectional drug delivery through the tissue into the systemic circulation, or local delivery of mucosal vaccines (20–22). The TFCs are

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novel bilayer films composed of a pH-sensitive mucoadhesive layer and a pharmaceutical wax as an impermeable backing layer. The mucoadhesive layer consists of Noveon® AA-1, a crosslinked polyacrylate polymer, and Eudragit S-100, a pH-sensitive anionic film-forming polymer composed of polymethacrylic acid-co-methyl methacrylate in a 3:1 (w/w) ratio. The pharmaceutical wax is DENTSPLY® Utility Wax. The films are made by first casting and drying an ethanolic gel of the mucoadhesive polymers to create a monolayer thin film and then by coating with the melted wax combined with other excipients using a proprietary process. We have developed a composition and method to permanently adhere the two layers together. It has been shown that both the adherence and erosion time of the mucoadhesive films can be controlled by altering the weight ratio of Noveon/Eudragit, the weight ratio of mucoadhesive/wax layer, and the thickness of each layer (20–22). Using this TFC, testosterone has been successfully delivered in rabbits, resulting in a relative bioavailability of 50.2 ± 3.2% without any absorption enhancer applied (22). When plasmid DNA vaccine was postloaded on the mucoadhesive side of the TFCs and applied on the buccal mucosa of rabbits, specific serum IgG to the expressed model antigen, β-galactosidase, and splenocyte proliferative immune responses were elicited (20,21).

In the present studies, we sought to investigate the feasibility of delivering sCT through rabbit buccal mucosa using these novel TFCs.

MATERIALS AND METHODS

Materials

Polycarbophil (Noveon® AA1) was a gift from BF Goodrich (Charlotte, NC, USA). Eudragit® S-100 was a gift from Rohm America, Inc. (Piscataway, NJ, USA). DENTSPLY® Utility Wax was purchased from DENTSPLY International Inc. (York, PA, USA). Ethanol and Tragacanth were from Spectrum Laboratory Products, Inc. (New Brunswick, NJ, USA). Synthetic salmon calcitonin was purchased from the American Peptide Company, Inc. (Sunnyvale, CA, USA). An ELISA kit for salmon calcitonin (EIAH-6003) and C18 Sep-column (RIK-SEPCOL) were purchased from Peninsula Laboratories, Inc. (San Carlos, CA, USA).

Preparation of Mucoadhesive Bilayer TFCs and Postloading of sCT

Mucoadhesive bilayer TFCs were prepared as previously described (20,21). The mucoadhesive layer consisted of Noveon/Eudragit S-100 in a ratio of 3:1 (% w/w). The mucoadhesive films were coated with melted DENTSPLY® Utility Wax containing 1% (w/w) tragacanth. Circular bilayer TFCs (3/8-inch in diameter) were punched from the larger films using Arch punches (C.S. Osborne and Co., Harrison, NJ, USA) and stored in ambient conditions away from light. sCT (40 μg in 40 μL of water) was postloaded on the 3/8-inch placebo TFCs by carefully dripping the solution on the mucoadhesive side of the films and allowed to dry at ambient conditions.

In Vitro Release of sCT from the TFCs

The release of sCT from TFCs was investigated by submerging the 3/8-inch diameter bilayer films (n = 3) into 200 mL of 10 mM phosphate-buffered saline (PBS) buffer (pH 7.4) in a plastic container with cap. The container was kept at 37°C in a C76 Water Bath Shaker (New Brunswick Scientific, New Brunswick, NJ, USA) rotating at 120 rpm. One milliliter of solution was withdrawn at specified times for sCT quantification using an ELISA kit from Peninsula Laboratories (EIAH-6003). Samples were diluted with the PBS if necessary. One milliliter of fresh 10 mM PBS buffer, pH 7.4, at 37°C was added to maintain a constant total volume immediately after sample withdrawing. Placebo films without sCT loading were used as a negative control.

Rabbit Studies

Twelve female New Zealand White Rabbits (2.8–3.0 kg) were obtained from Myrtle's Rabbitry (Thompson's Station, TN, USA). Rabbits were anesthetized by intramuscular (IM) injection of ketamine HCl (40 mg/kg) and xylazine (5 mg/kg). Anesthesia was maintained by IM injection of small doses of the ketamine/xzylazine mixture if needed. sCT (40 μg)-loaded TFCs were applied directly to the buccal pouch of the anesthetized rabbits (n = 6). After 180 min, the TFCs were removed. Six additional rabbits (n = 6) were injected via the ear vein with sCT (40 μg/0.5 mL of 0.2-μm filtered PBS, pH 7.4). Blood (volume recorded) was collected in citrated Vacutainer brand blood collection tubes at various times. Plasma was separated from whole blood by centrifugation at 960×g for 10 min and stored at −20°C until assayed.

Measurement of Plasma sCT Concentration

Plasma sCT was assayed using an enzyme-linked immunoassay (ELISA) kit (EIAH-6003) from Peninsula Laboratories. Plasma samples containing sCT were passed through C18 Sep Columns before being analyzed by ELISA. The extraction procedure used was as recommended by the Peninsula Laboratories except that the final extract was lyophilized. This extraction step was added to remove rabbit IgG from the plasma to avoid the precipitation of rabbit IgG in the serum samples with goat anti-rabbit IgG coated on the EIAH plates (14).

Quantitation of Plasma Calcium

Plasma calcium was quantified using an *o*-cresolphthalein complexone assay using a diagnostics kit for calcium (Procedure No. 587; Sigma, St. Louis, MO, USA). Calcium was detected at 575 nm by colorimetric determination (14).

Data and Statistical Analyses

The *in vitro* release of sCT from the TFCs was modeled using the equation $M = kt^n$ (23), where M is the fraction of total sCT released and t is time. The parameter k is the kinetic constant, incorporating the structural and geometric characteristics of the release system. From the value of exponential component (n), the mechanism of the *in vitro* release was determined. The computer software program Scientist® from MicroMath (Salt Lake City, UT, USA) was used to fit the data.

Pharmacokinetic parameters were determined using WinNonlin® Professional Version 3.1 from Pharsight Corporation (Mountain View, CA, USA) using noncompartmental

modeling (NCA Model 200) and the linear trapezoidal method. For the IV administration, mean residence time (MRT), C_{max}, $AUC_{0\text{-}240\ min}$, $AUC_{0\text{-}\infty}$, $AUMC_{0\text{-}240\ min}$, and $AUMC_{0\text{-}\infty}$ were generated by the WinNonlin software. For the buccal route, only the C_{max}, T_{max}, $AUC_{0\text{-}400\ min}$, and $AUMC_{0\text{-}400\ min}$ were generated. Other apparent pharmacokinetic parameters were calculated from the following equations: Systemic Clearance (Cl_s) = $dose_{iv}/AUC_{0\text{-}\infty}$, Volume of distribution at steady state (Vss) = $dose_{iv} \times AUMC/AUC^2$, elimination rate constant (k_e) = 1/MRT, elimination half-life ($t_{1/2}$) = 0.693/ k_e. The bioavailability (F) for the buccal route was calculated from the equation: $F = AUC_{0\text{-}400\ min\ [buccal]}/AUC_{0\text{-}\infty\ [IV]}$. Doses for the IV and buccal administrations were the same. Cl_s from the buccal route was calculated using equation: $Cl_s = F \times dose_{[buccal]}/ AUC_{0\text{-}400\ min\ [buccal]}$ (24).

The pharmacologic response to sCT was determined by analysis of plasma calcium concentrations. A baseline calcium concentration was obtained before sCT administration. The plasma calcium concentrations at each point were subtracted from the baseline concentration. The area above the calcium reduction curve (AACaC) was calculated with the linear trapezoidal method (24). AACaC from 0 to 240 min was only calculated for the purpose of comparison between IV and buccal routes because the last time point for the IV route was 240 min.

Except where mentioned, all statistical analyses were completed using a one-way analysis of variance followed by pairwise comparisons with Fisher's protected least significant difference procedure. A p value of ≤ 0.05 was considered to be statistically significant.

RESULTS

A previously developed TFC was used to postload sCT for buccal transmucosal delivery in rabbits. The 3/8-inch placebo TFCs weighed 9.7 ± 1.2 mg relative standard deviation ((RSD) = 12.4%) and had an overall thickness of 109 ± 6 μm (RSD = 5.5%). For the placebo TFCs, Noveon® AA-1 and Eudragit S-100 in the mucoadhesive layer composed about 60% of the total weight of the TFCs whereas the wax backing layer composed the other 40%. sCT (40 μg) was loaded on the mucoadhesive side of the preformed 3/8-inch TFCs and remained stable as measured by both ELISA and by the bioassay for reduction in plasma calcium levels.

The *in vitro* release profile of sCT from the TFCs is shown in Fig. 1. The profile showed a very slow release at the beginning followed by rapid and nearly zero-order release. Within 2 h, about 80% of the sCT was released. After fitting the first 60% of the sCT release data into the model of $M = kt^n$, a $k \times 10^{-4}$ value of 3.53 ± 2.12 and n value of 2.23 ± 0.18 were obtained.

sCT was detected in all rabbit plasma samples after both IV and buccal delivery. The mean plasma sCT concentration as a function of time after IV injection and buccal delivery using 3/8-inch diameter TFCs are shown in Fig. 2. Similar to typical IV bolus administration, the IV profile for sCT in the present study quickly reached a C_{max} followed by rapid elimination of sCT. A noncompartmental model was used to fit the data, and some of the relevant pharmacokinetic parameters are listed in Table I. Rabbits injected IV (n = 6) had C_{max} and $AUC_{0\text{-}\infty}$ values of 75.1 ± 6.5 ng/mL and 1925 ± 237 ng*min/mL, respectively. Rabbits (n = 6) dosed with sCT in

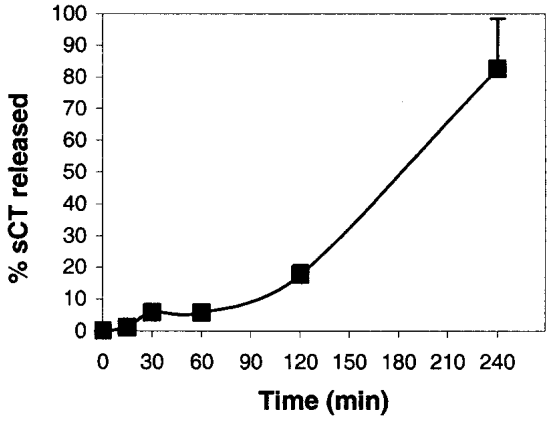

Fig. 1. *In vitro* release profile of sCT (40 μg) from 3/8-inch TFCs (n = 3) in PBS (10 mM), pH 7.4, at 37°C. Line through the mean values is included to show the trend of release, and it does not represent the mathematical fit of the data. Data are the mean ± SD.

TFCs had C_{max} and $AUC_{0\text{-}400\ min}$ values of 4.6 ± 1.6 ng/mL and 842.9 ± 209.7 ng*min/mL, respectively. Other relevant pharmacokinetic parameters for rabbits dosed with sCT via the buccal route are listed in Table II. A sustained therapeutic level of sCT in rabbit plasma was achieved using the TFCs. The relative bioavailability of sCT in rabbits treated with TFCs was 43.8 ± 10.9% with a CV of 24.9%.

Finally, for both IV and buccal routes, the plasma calcium levels were reduced after sCT administration (Fig. 3), demonstrating the pharmacologic activity of sCT in the rabbit. For both routes, a minimum calcium concentration was reached 120–150 min after dosing. The area above the calcium reduction curve ($AACaC_{0\text{-}240\ min}$) for IV and buccal routes were 546 ± 54 and 577 ± 226 mg*min/dL, respectively (Tables I and II). Statistical analysis showed no significant difference between the $AACaC_{buccal}$ and $AACaC_{iv}$ for the first 240 min (p = 0.37, two-sample t test assuming unequal variances).

DISCUSSION

In the present studies, using a previously developed novel mucoadhesive bilayer TFC, sCT was delivered through

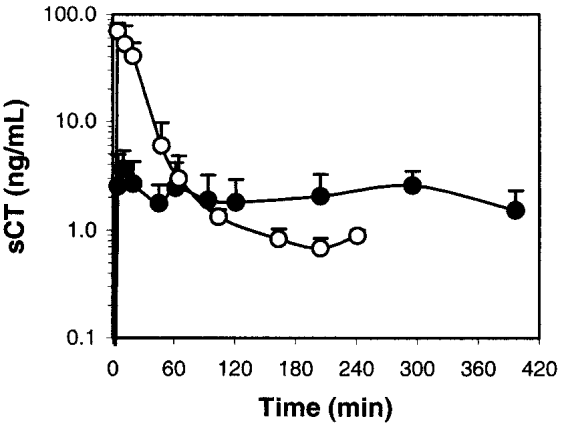

Fig. 2. Plasma sCT concentration vs. time profile in rabbits after IV injection (○) or buccal delivery of using 3/8-inch diameter TFCs (●). The dose of salmon calcitonin was 40 μg. All data are the mean value ± SD (n = 6). Lines do not represent the mathematical fit of the data. The therapeutic window for sCT is 0.1–0.4 ng/mL (14).

Table I. Mean Pharmacokinetic Parameters after IV Administration of sCT to Rabbits (n = 6, Mean ± SD)

Pharmacokinetic parameters	
$AUC_{0-240\ min}$ (ng · min/mL)	1882 ± 54
$AUC_{0-\infty}$ (ng · min/mL)	1925 ± 237
C_{max} (ng/mL)	75.1 ± 6.5
Cl_s (mL/min)	20.7 ± 3.3
V_{ss} (mL)	637 ± 141
MRT	29.3 ± 2.8
k_e (L/min)	0.034 ± 0.003
$t_{1/2}$ (min)	20.3 ± 1.9
$AACaC_{0-240\ min}$ (mg · min/dL)[a]	546 ± 54

[a] AACaC = Area above calcium reduction curve.

Table II. Mean Pharmacokinetic Parameters after Buccal Mucosal Application of sCT to Rabbits (n = 6, Mean ± SD)

Pharmacokinetic parameters	
$AUC_{0-400\ min}$ (ng · min/mL)	842.9 ± 209.7
C_{max} (ng/mL)	4.6 ± 1.6
T_{max} (min)	10.0 ± 5.4
F (%)	43.8 ± 10.9
Cl_s (mL/min)	22.0 ± 5.9
$AACaC_{0-240\ min}$ (mg · min/mL)[a]	577 ± 226

[a] AACaC = Area above calcium reduction curve.

rabbit buccal mucosa with a bioavailability of 43.8 ± 10.9%. In addition, the pharmacologic activity of sCT delivered by the buccal route, as measured by the area above calcium reduction curve (AACaC) for the first 240 min, was comparable with that after IV injection.

For diseases of calcium homeostasis, such as Paget's disease and osteoporosis, chronic treatment with CT is required (4). The chronic treatment necessitates the daily injection of a sterile product. Formulation of sCT for intranasal delivery is an alternative to the parenteral formulations. However, without any permeation enhancer, a bioavailability of only 1.6% was reported for a 200-IU commercial formulation of sCT in human volunteers (9). With 0.5% sodium tauro-24, 25-dihydrofusidate, the highest bioavailability obtained was 7.9% (205-IU administered) (9). These results clearly suggest the need for alternative routes of delivery for sCT. The oral buccal mucosa, as a viable route to deliver sCT, has been investigated by several research groups (12–14). Nakada *et al.* (12) studied the buccal delivery of calcitonin in rats and reported a 1.6% decline in plasma calcium levels, indicating the successful delivery of pharmacologically active sCT through the buccal mucosa. However, no bioavailability information was provided. Heiber *et al.* (13) reported the delivery of a total of 550-IU (0.11 mg) of sCT through dog buccal mucosa using a tablet formulation. Their results demonstrated that therapeutic levels of sCT could be achieved by the buccal route, although again, the authors did not provide relative bioavailability data. More recently, Alur *et al.* (14) evaluated the delivery of sCT (40 μg or 200-IU) in rabbits using an alternative buccal tablet. The highest apparent bioavailability reported was 37 ± 6%. Without the addition of any chemical penetration enhancer to the TFCs, a bioavailability of 43.8 ± 10.9% was achieved in the present studies. This bioavailability strongly points out the potential of the buccal mucosa as a viable route for sCT delivery. Still, the bioavailability for most peptides by the buccal route remains low and often requires the use of enhancers. For example, the estimated bioavailability for TRH in rats, hybrid α-interferon in rats, and Octreotide in dogs was only 2%, 0.014%, and 0.3-2%, respectively (9).

Alur *et al.* (14) applied a two-compartmental model to describe the plasma sCT concentration vs. time profile after IV injection of sCT in rabbits (14). They reported Cl_s of 19 ± 2 (mL/min*kg) and V_d (β) of 1484 ± 454 (mL/kg). Sinko *et al,* (24), using a noncompartmental model to fit the plasma profiles in rats after IV injection of sCT, reported the following pharmacokinetic parameters (mean value of three different doses): Cl_s = 2.58 ± 0.34 (mL/min), V_{ss} = 140 ± 10 (mL), MRT = 57.7 ± 11.3, k_e = 0.02 ± 0.002 (1/min), and $t_{1/2}$ = 40.0 ± 7.8 (min) (24).

In the present studies, plasma sCT vs. time data for both the IV and buccal routes were fitted into noncompartmental model. For the buccal profile, only the $AUC_{0-400\ min}$ could be accurately estimated. Therefore, both bioavailability (F) and Cl_s were calculated using truncated data. The F value of 43.8% most likely underestimated the bioavailability from the TFC system. The calculated Cl_s value of 22.0 ± 5.9 (mL/min) for the buccal route was comparable with the value of 20.7 ± 3.3 (mL/min) for the IV route (p = 0.47, two-sample t test assuming equal variances). Unlike that for the IV injection, the plasma sCT concentration for the buccal route reached C_{max} within 10 to 15 min and was maintained above therapeutic levels (0.1–0.4 ng/mL) for a sustained duration, demonstrating that a sustained delivery of sCT was achieved by the buccal route. These data agree with that of Heiber *et al.* (13) and Alur *et al.* (14) using sCT-loaded buccal tablets in dogs and in rabbits, respectively. The mechanisms for achieving the high bioavailability and sustained delivery of sCT in the present study are currently unknown. It is possible that the slowly swelling mucoadhesive films, when adhered to the buccal mucosa, successfully prevented the release of sCT into the oral cavity and therefore prevented the degradation of sCT by local enzymes. Furthermore, it was thought that sCT may be released unidirectionally into the buccal mucosa from

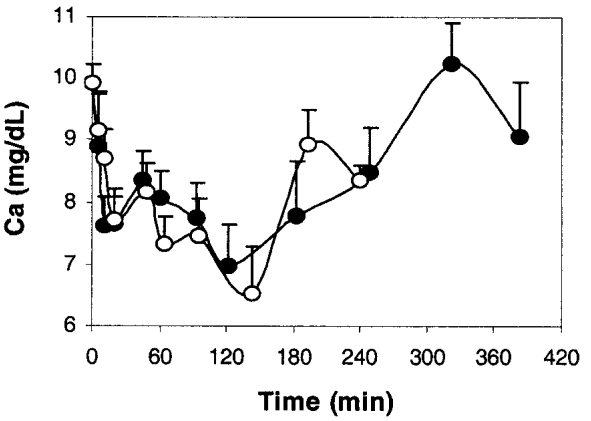

Fig. 3. Plasma calcium concentration vs. time profiles in rabbits after delivery of salmon calcitonin either by intravenous injection (○) or using 3/8-inch sCT-loaded TFCs (●). All data showed are mean value ± SD (n = 6). Lines do not represent the mathematical fit of the data. The normal calcium level in plasma is 10 mg/dL (14).

the wax-backed TFCs resulting in a sCT reservoir for enhanced and sustained transmucosal absorption.

Similar to the results reported by Alur *et al*. (14), the calcium level in the plasma of rabbits in the present study was reduced below the baseline level (10.0 mg/dL) after both IV and buccal administration. After reaching a minimum at 120–150 min, the calcium level seemed to begin to return to baseline levels. In contrast, Alur *et al*. (14) showed that the plasma calcium levels did not return to baseline levels within 120 min after IV injection or almost 300 min after application of buccal sCT tablets. Alur *et al*. explained that, in the case of buccal tablet administration, this is probably caused by the reservoir effect of sCT in the buccal mucosa. This explanation was supported with the observation that the plasma sCT level was maintained even after the tablets were removed at 180 min. However, Alur *et al.* (14) likely did not monitor plasma calcium levels long enough because their last measurement after IV and buccal administration was only at 120 min and 300 min, respectively. In the present study, the reduction in plasma calcium levels after IV and buccal administration was over 240 min and close to 400 min, respectively. We further speculated that the observed elevation of calcium level after the minimum point might be caused by the inactivation of sCT in the plasma over time. The plasma sCT concentration was determined using ELISA, which may detect peptide whether it is biologically active or inactive. Therefore, it is possible that although a sustained level of sCT was present in the plasma, it was no longer biologically active. Alternatively, it is also possible that calcium levels returned to normal levels even in the presence of circulating intact sCT because of a biologic feedback mechanism for calcium (14,24). In the present study, it was found that the areas above the calcium reduction curve for both IV injection and buccal application in the first 240 min were comparable, although the C_{max} for the IV route was 16-fold higher than the C_{max} for the buccal route. This result may be related to the minimum amount of sCT needed to induce the maximum pharmacological response as reported by others (24). Specifically, the reduction of plasma calcium level is not directly proportional to the plasma concentration of sCT. In fact, Sinko *et al*. (24) estimated that the maximal calcium lowering effect occurs at a sCT concentration as low as 10 pg/mL in rats. Because of this, the authors cautioned the use of AACaC to predict the oral bioavailability of sCT.

The *in vitro* release profile of sCT showed slow release at the beginning following by more rapid and close to zero-order release thereafter. After fitting the data into the model of $M = kt^n$, the value for the diffusional exponent n was found to be 2.23 ± 0.18, strongly indicating a Super Case-II transport mechanism for the release of sCT from the TFCs (25). In the postloading of sCT on the TFCs, it is likely that most of the sCT was entangled with the polymers in the mucoadhesive film. It would be difficult for the sCT molecules to release from the film before hydration of the polymers and swelling of the mucoadhesive film. Therefore, a slow release, which might be caused by the slow diffusion of some of the sCT loosely associated on the surface of the film, was observed at the beginning. One of the components of the mucoadhesive film, Noveon® AA1 or polycarbophil, is a homopolymer of acrylic acid cross-linked with divinyl glycol. The high molecular weight polycarbophils readily swell in water and biologic fluids (26). When the polymers were hydrated and the film was swelled (i.e., polymer relaxation), the release of sCT was then mainly due to drug diffusion mechanism (23), demonstrated by the close to zero-order release of sCT.

In conclusion, a relative bioavailability of 43.8 ± 10.9% was achieved after buccal application of sCT-loaded TFCs in rabbits. The resulting reduction in plasma calcium levels was comparable with that after conventional IV injection of sCT.

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