Evaluation of a Novel, Natural Oligosaccharide Gum as a Sustained-Release and Mucoadhesive Component of Calcitonin Buccal Tablets

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Abstract □ The objective of this study was to evaluate the gum from *Hakea gibbosa* (hakea) as a sustained-release and mucoadhesive component in buccal tablets for a model peptide, namely, salmon calcitonin. Flat-faced core tablets containing either 12 or 32 mg of hakea and 40 μg (200 IU) of salmon calcitonin (sCT) per tablet were formulated using a direct compression technique and were coated with Cutina on all but one face. The in vitro release profiles were sigmoidal in nature and according to a mathematical model indicated super Case II transport as the primary mechanism of release. The resulting plasma sCT and calcium concentrations were determined following both intravenous administration and buccal application of mucoadhesive tablets in rabbits. Following intravenous administration, the mean values determined for $t_{1/2}$ (α), $t_{1/2}$ (β), V_d, and CL for sCT were 0.76 ± 0.06 min, 67 ± 18 min, 1484 ± 454 mL/kg, and 19 ± 2 mL/min·kg, respectively. Following the application of the mucoadhesive buccal tablets which contained 40 μg of sCT and either 12 or 32 mg of hakea, the calculated apparent bioavailability (*F*) and clearance (CL) were 37 ± 6% and 19 ± 3.3 mL/min·kg and 16 ± 8% and 18 ± 0.4 mL/min·kg, respectively. Serum calcium concentrations indicated that biologically active sCT was delivered across the rabbit buccal mucosa. The strength of mucoadhesion of the tablets was also quantitated in terms of the force of detachment as a function of time. The force of detachment for the mucoadhesive buccal tablets containing either 12 or 32 mg of hakea and 40 μg of sCT increased from 4.47 ± 0.68 to 8.41 ± 1.0 N and 8.23 ± 1.62 to 14.98 ± 1.63 N, respectively, from 5 to 90 min following application to excised rabbit intestinal mucosa. These results demonstrate that the novel, natural gum from *Hakea gibbosa* may be used to sustain the release of sCT from a unidirectional-release buccal tablet. The mechanism of in vitro release is likely to involve peptide diffusion/polymer dissolution. The mucoadhesive strength, as measured by the force of detachment, can be modulated by altering the amount of hakea in the tablet. The mucoadhesive buccal tablets described in this paper represent an improved transbuccal delivery system for therapeutic polypeptides.

Introduction

Proteins and peptides are currently emerging as a major class of future therapeutic drugs. With the advent of and improvement in techniques like solid-phase synthesis, combinatorial chemistry, and protein biotechnology, more and more proteins and peptides are being made available in large quantities. Pharmaceutical scientists are facing increasing challenges with respect to the formulation of new and novel delivery systems and exploring new routes to successfully deliver these bioactive agents. Noninvasive delivery of proteins and peptides has been met with limited success for a variety of reasons[1,2] including (a) low permeability due to hydrophilicity, globular structure, and size, (b) inactivation by enzymes at the site of delivery or absorption prior to reaching the systemic circulation, and (c) short residence time of the drug as well as the delivery system at the site of absorption, all leading to subtherapeutic levels in the systemic circulation.

Calcitonin (CT) is a single-chain 32 amino acid polypeptide with a disulfide bridge between cysteine residues at positions 1 and 7 and has a molecular weight of 3432 Da. The hormone is secreted by the parafollicular cells of the thyroid glands in mammals and by the ultimobronchial glands in birds and fishes. CT functions as a hypocalcemic agent by inhibiting bone resorption and reducing the renal tubular reabsorption of calcium, and it is used in the treatment of Paget's disease, hypercalcemia, and osteoporosis.[3,4] Treatment calls for daily or alternate day subcutaneous or intramuscular injections for an extended period. This is bothersome and inconvenient for patients. Salmon calcitonin (sCT) is one of the calcitonins and is currently available in a sterile solution for injection and nasal spray form. Lee et al.[5] reported to have developed biodegradable porous PGA microspheres of sCT which provide sustained levels of sCT for over 5 days following a single subcutaneous (sc) injection. The buccal mucosa has been investigated recently for the delivery of this bioactive peptide.[6] Buccal mucosa is an easily accessible tissue, which is less sensitive to irritation or irreversible damage.[7] It also provides drug delivery by avoiding the harsh environment of the gastrointestinal tract and first-pass metabolism.[7]

The risk of accidental swallowing and salivary washout limits the use of solutions or conventional buccal tablets, and it has led to the development of self-adhesive buccal tablets as the preferred dosage form.[8] Mucoadhesive dosage forms retain the dosage form in intimate contact with the mucosa (absorbing membrane) thereby increasing the total amount of drug which penetrates the mucosa.[9]

Previous studies[10,11] have shown the efficacy of a novel, natural gum from *Hakea gibbosa* as a sustained-release and mucoadhesive component in mucoadhesive buccal tablets for the delivery of a low molecular weight organic compound. Thus, the present study was undertaken to investigate the possibility of using this novel excipient as a sustained-release and mucoadhesive component of buccal tablets for peptide and protein delivery. Hakea gum is a polysaccharide exudate from the tree *Hakea gibbosa* (Family: Proteaceae). The trees are indigenous to New South

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Wales, Australia. The gum has a molecular weight of greater than 2×10^6 and is totally water soluble at a concentration of 2% w/v. The gum is composed of sugars such as galactose, mannose, xylose, arabinose, and glucuronic acid. The chemical structure of the gum has been previously reported.[10,12]

Materials and Methods

Materials—Salmon calcitonin sCT (5001 IU/mg) was a generous gift from Rhone-Poulenc-Rorer (Vitry Sur Seine Cedex, France). All solutions were prepared in deionized distilled water. The hakea gum was a gift from Dr. Peter Eagles of the University of the Western Cape, Cape Town, South Africa. The gum was obtained from the Kirstenbosch Botanical Gardens. All other materials, except for the gum, were used as received. The gum was purified by first dissolving it in water and then filtering the 2% solution through muslin cloth. The filtered solution was freeze-dried using a model 10-MR SAVirtis tabletop freeze drier (Gardiner, NY).

Cutina (castor oil, hydrogenated) was obtained from Henkel Corporation, NJ. Xylazine (100 mg/mL), ketamine (100 mg/mL), and pentobarbital sodium (50 mg/mL) solutions were provided by the Laboratory Animal Center at the University of Missouri—Kansas City (Kansas City, MO). Heparin sodium injection (10000 units/mL) was purchased from Elkins-Sinn, Inc. (Cherry Hill, NJ). I-Cath intravenous placement units with stylet attachment (catheter, 22G × 12 in.; needle 19G × 21 in.) was purchased from Charter Med, Inc. (Lakewood, NJ), and tuberculin syringes (1 cc) were obtained from Becton and Dickinson (Sandy, UT).

Blood samples were collected into 1.5 mL Eppendorf tubes containing heparin sodium (100 units/mL) and centrifuged using a Beckman GS-15R centrifuge (Palo Alto, CA).

Male New Zealand albino rabbits, weighing between 2.0 and 2.5 kg, were purchased from Myrtle's rabbitry (Thompson, TN). The animals were housed individually for at least 1 week prior to experimentation and allowed food and water ad libitum. The average weight of the rabbits at the time of the study was 2.8 ± 0.3 kg ($n = 9$).

The bioadhesion experiments were carried out on a model LTC universal tension-compression stand (John Chatillon and Sons, Inc., Greensboro, NC) equipped with a model DFM-10 digital force gauge (John Chatillon and Sons, Inc., Greensboro, NC).

Methods—*Tablet Preparation*—Flat-faced core tablets were prepared by direct compression, and the tablets were coated with Cutina on all but one face using a compression coating technique. Release of sCT was unidirectional, occurring from only the uncoated tablet face.

(a) Direct Compression—The tablets were prepared by initially mixing the sCT and hakea for 10 min. Subsequently, spray-dried lactose (hydrous N.F. grade, Fast-Flow 316) and Cab-o-sil (M-5 grade, amorphous fumed silica) were incorporated and the powder was mixed for an additional 10 min. Finally, magnesium stearate was added and the mixing continued for an additional 5 min. Mixing was performed by mechanical rotation at 225 rpm.

(b) Tablet Compression—Both core and coated tablets were prepared on a model B, No. 0-24R carver press (Summit, NJ). The core tablets (weight: 101.5 mg) had a diameter of 1 cm and a thickness of 0.1 cm and were compressed at a force of 5000 psi. The coated tablets (final weight: 202.5 mg) were compressed at 2000 psi force to generate a final diameter of 1.2 cm and a thickness of 0.2 cm.

(c) Release Study—The in vitro release studies were performed using jacketed glass vessels (250 mL) obtained from Fisher Scientific (Pittsburgh, PA). The dissolution medium consisted of 200 mL of deionized water (pH 7.0) at 37 °C. The dissolution medium was stirred with a magnetic stir bar at a speed of 50 rpm. Sink conditions were maintained throughout the duration of release study. The in vitro release samples (1 mL) were analyzed for sCT using an enzyme immunoassay kit (EIAH-6003) obtained from Peninsula Laboratories (Belmont, CA) after appropriate dilutions were made with deionized water. The release studies were carried out for 90, 420, and 600 min for buccal tablets containing 40 μg of sCT and 0, 12, and 32 mg of hakea, respectively.

Animal Preparation—The animals were prepared for both intravenous and buccal sCT studies by anesthetizing with an im injection of a 1:5 mixture of xylazine (1.9 mg/kg) and ketamine (9.3 mg/kg). Following induction of anesthesia, a catheter was placed in the marginal ear vein for blood sample collection. After the collection of each sample, the cannula was flushed with 0.2 mL of a 10% (v/v) heparin/normal saline solution to keep the cannula patent. A light plane of anesthesia was maintained by an im injection of one-third of the initial dose of xylazine and ketamine mixture as needed. All the blood samples were centrifuged at 3000 rpm for 10 min to separate the plasma and the retrieved plasma, was stored at −20° C until the time of analysis. At the end of the experiments the rabbits were euthanized by injecting an overdose of pentobarbital solution (3−5 mL) into the catheter.

The research adhered to the principles of Laboratory Care (NIH publication no. 85-23, revised 1985), and the animal protocol was approved by the animal care and use committee of the University of Missouri—Kansas City.

Intravenous sCT Study—Following the induction of anesthesia, sCT (40 μg) was administered as a bolus through the cannulated ear vein of the rabbit. A blood sample (2 mL) was obtained 5 min before and then at 5, 10, 20, 45, 60, 90, and 120 min following the injection.

Buccal sCT Study—Upon the induction of anesthesia, a tablet was applied to the buccal mucosa of the rabbit by pressing it firmly against the mucosa for 1.5 min. A drop of water was placed on the releasing face of the tablet before it was applied to the buccal mucosa. A blood sample (2 mL) was obtained 5 min before and then at 5, 10, 20, 45, 60, 90, 120, and 180 min following the application of the mucoadhesive buccal tablets containing 40 μg of sCT and 12 mg of hakea. The tablet was removed at 180 min, and additional blood samples were obtained at 210, 240, 270, and 300 min following the removal of the buccal tablet. For tablets containing 40 μg of sCT and 32 mg of hakea, a similar blood sampling protocol was followed until 180 min as with 12 mg hakea.

Quantitation of Plasma sCT—Plasma samples were analyzed for sCT using an enzyme immunoassay kit (EIAH-6003) obtained from Peninsula Laboratories (Belmont, CA). The sensitivity of the assay ranged from 0 to 25 ng/mL. The assay was linear over the range 0.04−2.0 ng/mL. The sCT contained in the samples was extracted using C18 Sep columns (Peninsula Laboratories Belmont, CA) before being analyzed by enzyme immunoassay. The reason for the inclusion of an extraction step was to avoid a precipitation reaction between rabbit IgG present in the samples and goat anti-rabbit IgG coated on the EIAH plates. The extraction procedure was obtained from Peninsula Laboratories (Belmont, CA) and was followed without any modification. The samples were appropriately diluted with the EIA buffer prior to analysis.

Quantitation Of Plasma Calcium—Plasma calcium was quantitated by the *o*-cresolphthalein complexone method using a Sigma diagnostics kit for calcium (Sigma, St. Louis, MO). The method involves colorimetric determination of calcium at 575 nm (Procedure No. 587, Sigma Diagnostics).

Bioadhesion Study—Rabbit small intestine was selected as a model membrane since the intestine provided a flat and uniform surface and the surface area of the buccal mucosa in a rabbit is only slightly larger than the buccal tablets evaluated in these studies. A 2 cm long piece of intestinal mucosa was mounted on the platform of the tension-compression stand. The tablet was applied using super glue to the bottom face of a stainless steel disk attached to the force gauge. The mucosal surface was hydrated by placing 20 μL of distilled water on the tissue surface. The tablet and the mucosal surfaces were brought into contact, and a constant force of 20 N was applied. The tablet was pulled off the tissue surface at 5, 10, 20, 30, 45, 60, and 90 min following application of force. The value for the force of detachment was measured in newtons by lowering the platform of the tension-compression stand at a constant rate of 1 mm min^{-1}.

Data And Statistical Analysis—The in vitro release from the unidirectional buccal tablets was modeled using the basic equation $F = kt^n$,[13] and from the values of n, the mechanism of in vitro release was determined.

The plasma sCT concentrations obtained after both intravenous and buccal administration were not corrected for baseline levels of sCT (~0.41 ± 0.21 ng/mL, $n = 6$) for the ease of comparison with previous published findings. The plasma sCT concentration versus time data obtained following intravenous administration

were fit to a polyexponential equation using the R STRIP program. The data were fit to a two-compartment model with elimination from the central compartment. The model-dependent phamacokinetic parameters AUC, A, B, $t_{1/2}$ (α), and $t_{1/2}$ (β) were obtained from the fit, whereas the volume of the central compartment (V_c) and the volume of distribution in the β-phase (V_d (β)) were obtained using standard equations.[14]

The area under the plasma sCT concentration versus time curve was calculated using the trapezoidal rule within the time periods of 0−2 h following buccal administration although the experiments were carried out beyond 2 h. In an attempt to estimate the apparent bioavailability, the data was truncated at 2 h following buccal administration because the total duration of the intravenous experiments was 2 h. Equations 1 and 2 were used to estimate the apparent bioavailability and clearance of sCT following buccal administration.[15,16] The C_{max}, C_{min}, and t_{max} were estimated directly from the plasma sCT versus time profiles following buccal administration. In eqs 1 and 2, F denotes the

$$F = \frac{(\text{dose}_{IV})\,(\text{AUC}_{0-t_{buccal}})}{(\text{dose}_{buccal})\,(\text{AUC}_{0-t_{IV}})} \quad (1)$$

$$\text{CL} = \frac{FD}{\text{AUC}_{0-t_{buccal}}} \quad (2)$$

apparent bioavailability, D is the dose of sCT contained in the buccal tablets, and $\text{AUC}_{0-t_{buccal}}$ represents the area under the plasma sCT concentration vs time curve following buccal administration from 0 to 2 h. A dose of 40 μg of sCT was used to calculate the apparent bioavailability as the amount of sCT remaining in the tablet after buccal administration was not estimated.

The area above the calcium reduction curve from 0 to 2 h (AAC) was obtained as described previously.[17,18] The plasma calcium concentration at each time point after the administration of sCT was subtracted from the baseline calcium concentration, which was obtained prior to administration of sCT. Next, the AAC was calculated using the trapezoidal method. The data was again truncated at 2 h following buccal administration for ease of comparison with the intravenous data.

All experiments were conducted in triplicate, and the results were expressed as the mean value ± the standard deviation. Mean values were compared for statistical significance at the 5% level using Student's one-tail t-test.

Results

In Vitro Release Study—In vitro release profiles from buccal tablets containing 0, 12, and 32 mg of hakea with 40 μg of sCT are shown in Figure 1. The release profiles are sigmoidal in nature for buccal tablets with 40 μg of sCT and either 12 or 32 mg of hakea. The mechanism of in vitro release was determined from the values of n obtained by modeling the first 60% of the release to the equation $F = kt^n$.[13] The values of k (kinetic constant) and n (diffusional exponent) are listed in Table 1. The values of n are greater than 1, indicating super Case II transport as the mechanism of sCT release.

Intravenous sCT Study—The plasma sCT concentration versus time profile following intravenous administration was best explained using a biexponential equation and is shown in Figure 2. The values of the model-dependent phamacokinetic parameters are listed in Table 2. The change in the plasma calcium concentration is also depicted in Figure 2, and the relative AAC_{0-2h} is listed in Table 2. It can be noted from the pharmacokinetic parameters that the extravascular distribution of sCT was very rapid.

Buccal sCT Study—The plasma sCT concentration vs time profiles following administration of the 40 μg sCT buccal tablets with either 12 or 32 mg of hakea are shown in Figures 3 and 4, respectively. The relevant pharmacokinetic parameters are listed in Table 3. From Figures 3 and 4, it is apparent that hakea effectively sustained the release of sCT from the buccal tablets and also maintained an elevated plasma sCT concentration during the entire application period. The apparent bioavailability (F) of sCT from the tablets with 12 mg of hakea was significantly ($p < 0.05$) greater than that from the tablets with 32 mg of hakea. The C_{max} and C_{min} decreased while the t_{max} increased with an increase in the amount of hakea contained in the tablet (Table 3). The change in the plasma calcium concentration has also been illustrated in Figures 3 and 4, and the mean values of AAC_{0-2h} are listed in Table 3. AAC_{0-2h} for sCT tablets with 12 mg of hakea was not significantly different ($p > 0.05$) from AAC_{0-2h} for tablets with 32 mg of hakea.

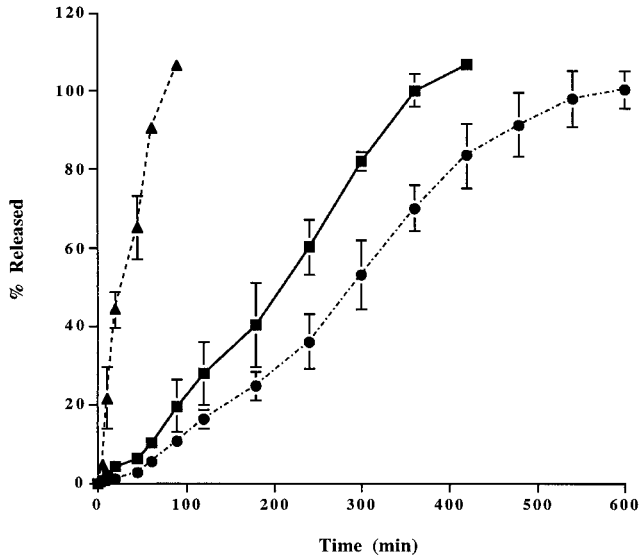

Figure 1—In vitro release profiles of sCT from directly compressed tablets which contained 40 μg of sCT and 0 mg of hakea (▲), 12 mg of hakea (■), or 32 mg of hakea (●). All data points represent the mean value ± standard deviation of three experiments. Lines through mean values are included to illustrate the trend and do not represent a mathematical fit of the data.

Table 1—Diffusional Exponent (n) and Kinetic Constant (k) Values[a]

parameters	40 μg of sCT and 12 mg of hakea	40 μg of sCT and 32 mg of hakea
n	1.24 ± 0.07	1.37 ± 0.05*
$k \times 10^{-7}$	2.0 ± 1.8	3.4 ± 1.3

[a] Mean ± SD, $n = 3$. The * symbol indicates a statistically significant increase in the mean value of the diffusional exponent when a 40 μg sCT tablet containing 12 mg of hakea was compared with a tablet containing 32 mg of hakea at $p < 0.05$ using Student's one-tail t-test.

Bioadhesion Study—A profile showing the mean values of the force of detachment of the sCT buccal tablets following their application to excised rabbit intestinal mucosa is shown in Figure 5. It can be noted that the mean values of the force of detachment increased with time and reached a plateau at later time points. The mean values of the force of detachment were significantly ($p < 0.05$) greater at each time point for tablets containing 32 mg of hakea when compared to the tablets which contained 12 mg of hakea and were significantly greater for tablets containing 12 mg of hakea when compared to the tablets which contained no hakea.

Discussion

In the present study, controlled release of sCT both in vitro and in vivo was successfully demonstrated using the natural gum, hakea as an excipient. The in vitro release profiles were sigmoidal in nature and consisted of an initial slow-releasing phase followed by a linear phase, where the

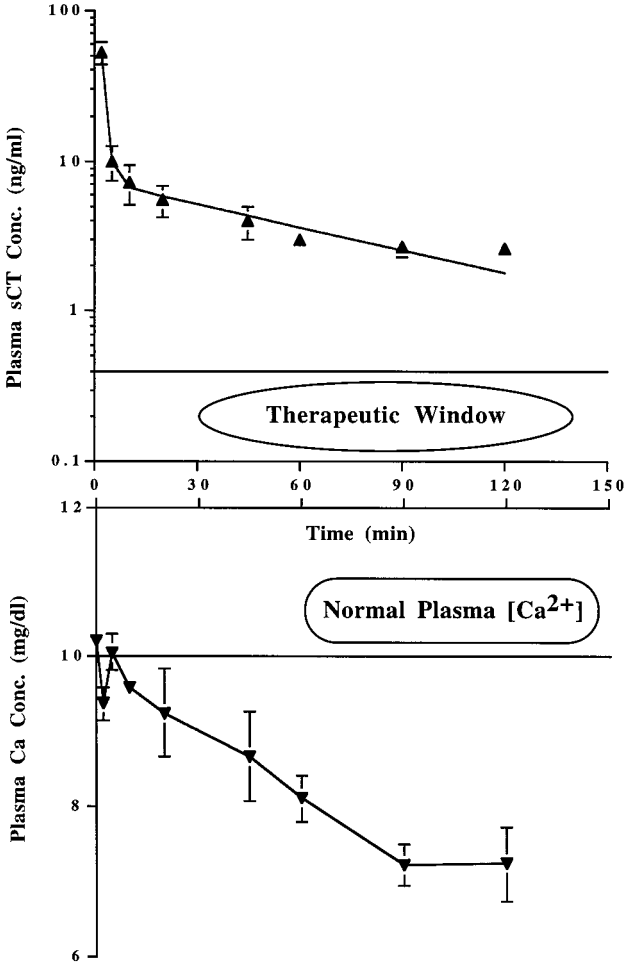

Figure 2—Plasma profiles of sCT (▲) and calcium (▼) in rabbits following intravenous administration of 40 μg (200 IU) of sCT. All data points represent the mean value ± standard deviation of three experiments. The line (—) represents the mathematical fit of the intravenous data. Therapeutic window of sCT = 0.1–0.4 ng/mL (from ref 6).

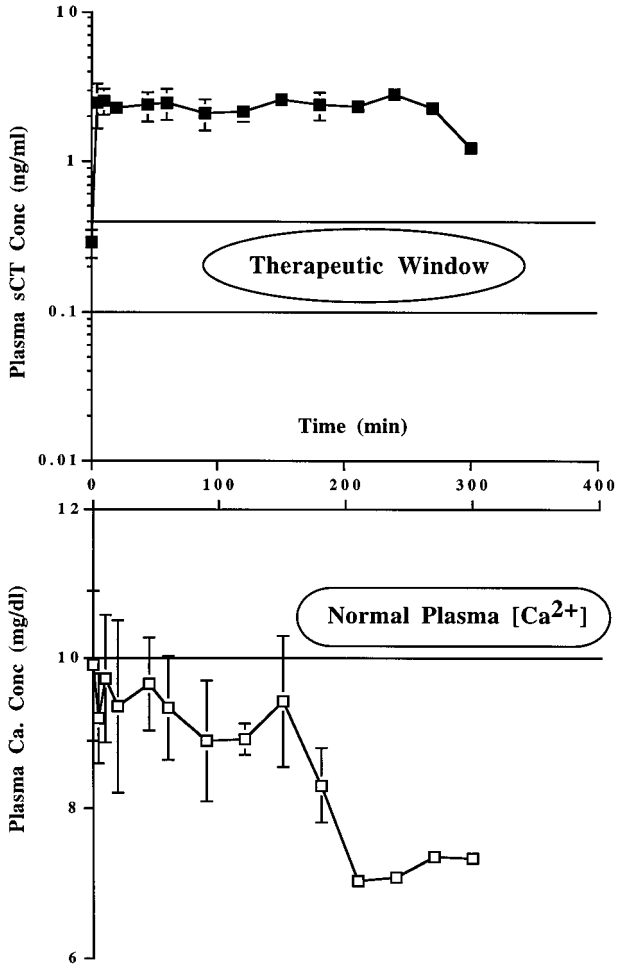

Figure 3—Plasma profiles of sCT (■) and calcium (□) in rabbits following the application of buccal tablets containing 40 μg (200 IU) of sCT and 12 mg of hakea. Buccal tablets were removed at 180 min. All data points represent the mean value ± standard deviation of three experiments. Lines through mean values are included to illustrate the trend and do not represent a mathematical fit of the data. Therapeutic window of sCT = 0.1–0.4 ng/mL (from ref 6).

Table 2—Pharmacokinetic Parameters[a] of sCT after Intravenous Bolus Administration of 40 mg of sCT in New Zealand Albino Rabbits for a Two-Compartment Open Model

pharmacokinetic parameters	
AUC_{0-2h} (ng·min/mL)	807 ± 119
$AUC_{0-\infty}$ (ng·min/mL)	1002 ± 171
CL (mL/min·kg)	19 ± 2
A (ng/mL)	290 ± 89
B (ng/mL)	7.6 ± 2.5
$t_{1/2}$ (α) (min)	0.76 ± 0.06
$t_{1/2}$ (β) (min)	67 ± 18
V_c (mL)	143 ± 43
$V_d(\beta)$ (mL/kg)	1484 ± 454
AAC_{0-2h} (mg·min/dL)[b]	208 ± 27

[a] Mean ± SD, $n = 3$. [b] Area above the calcium reduction curve.

release appeared to follow zero-order kinetics (Figure 1). The values for the diffusional exponent (n) were greater than 1 (Table 1), indicating that the mechanism of sCT release was super Case II transport.[19] That is, the release of sCT from the buccal tablets is likely due to the combination of polypeptide diffusion and polymer relaxation/dissolution. This mechanism also explains the initial slow-releasing phase, where the polymer was not completely hydrated, resulting in an incomplete relaxation of the side chains. Insufficient hydration would lead to the creation of an aqueous pore through which the free diffusion of sCT would be hindered. Upon complete hydration, the polymer presumably began to dissolve with subsequent relaxation of the side chains. Relaxation of hakea's numerous side chains would then allow free diffusion of sCT from the tablet matrix. This mechanism would tend to support zero-order release kinetics during the linear portion of Figure 1. Banga et al.[20] reported that the diffusion of three polypeptides, vasopressin, calcitonin, and insulin, from hydrogel formulations was dependent on their molecular size.

The intravenous profile in this study was best described by a two-compartment model which is consistent with the work of Heiber et al. using dogs[6] and Sinko et al. using rats.[18] Sinko et al.[18] using a noncompartmental pharmacokinetic analysis reported the values (mean value of three different doses) for CL, $t_{1/2}$, k_e, and V_{ss} of 2.58 ± 0.34 mL/min, 40 ± 7.8 min, 0.02 ± 0.002 min^{-1}, and 0.14 ± 0.001 L, respectively, in rats. Beveridge et al.[21] reported terminal elimination half-lives ranging between 60 and 90 min following iv, im, and sc administration of 35 μg of sCT to humans.

The tablets which contained 32 mg of hakea demonstrated a significantly ($p < 0.05$) lower mean value of the apparent bioavailability (F) compared to tablets which contained 12 mg of hakea. The C_{max} and C_{min} decreased while the t_{max} increased when the amount of hakea in the tablet was increased from 12 to 32 mg (Figures 2 and 3;

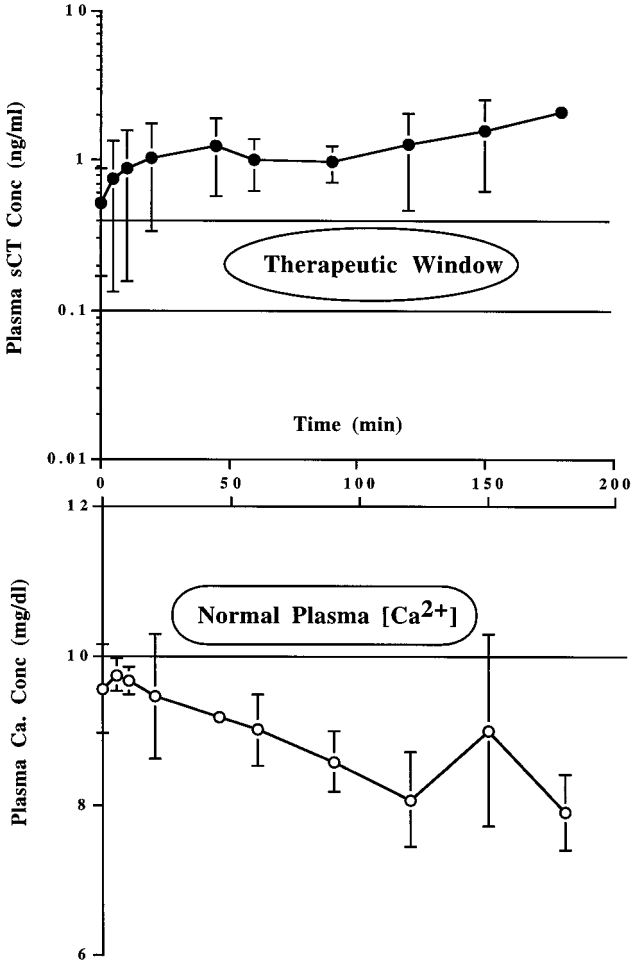

Figure 4—Plasma profiles of sCT (●) and calcium (○) in rabbits following the application of buccal tablets containing 40 μg (200 IU) of sCT and 32 mg of hakea. All data points represent the mean value ± standard deviation of three experiments. Lines through mean values are included to illustrate the trend and do not represent a mathematical fit of the data. Therapeutic window of sCT = 0.1–0.4 ng/mL (from ref 6).

Table 3—Pharmacokinetic Parameters[a] of sCT after Buccal Administration in New Zealand Albino Rabbits for a Two-Compartment Open Model

pharmacokinetic parameters	40 μg of sCT and 12 mg of hakea	40 μg of sCT and 32 mg of hakea
AUC_{0-2h} (ng·min/mL)	273 ± 49*	125 ± 63
CL (mL/min·kg)	19 ± 3.3	18 ± 0.4
F (%)	37 ± 6†	16 ± 8
C_{max} (ng/mL)	2.50 ± 0.5	1.33 ± 0.65
C_{min} (ng/mL)	2.00 ± 0.2	0.68 ± 0.41
t_{max} (min)	70 ± 17	140 ± 17
AAC_{0-2h} (mg·min/dL)[b]	74 ± 35	71 ± 59

[a] Mean ± SD, n = 3. The * symbol indicates a statistically significant increase in the mean value of the area under the curve from 0 to 120 min when a 40 μg sCT tablet containing 12 mg of hakea was compared with a tablet containing 32 mg of hakea at $p < 0.05$ using Student's one-tail t-test. The † symbol indicates a statistically significant increase in the mean value of the apparent bioavailability (F) when a 40 μg sCT tablet containing 12 mg of hakea was compared with a tablet containing 32 mg of hakea at $p < 0.05$ using Student's one-tail t-test. [b] Area above the calcium reduction curve.

Table 3). The results tend to suggest that hakea effectively retarded the rate of release of sCT from the dosage form. The values of F (%) calculated in the present study were 37 ± 6 and 16 ± 8 for buccal tablets with 12 and 32 mg of hakea, respectively. Dua et al.[22] reported percent bioavailability values of 0.16, 0.80, and 0.71 for low viscosity (1

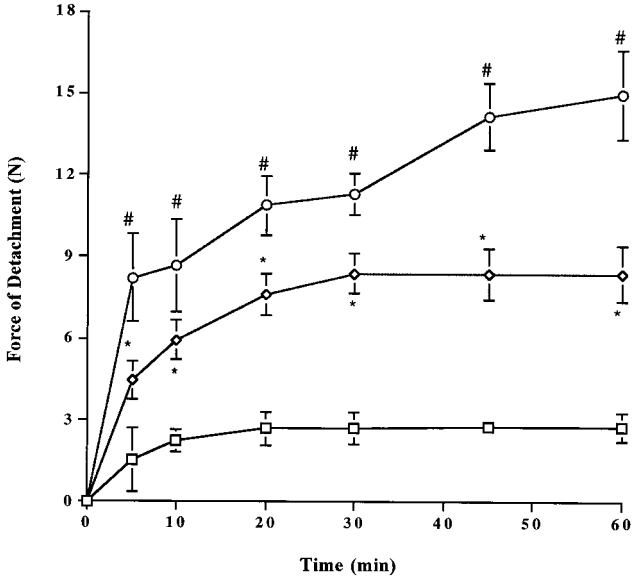

Figure 5—The force of detachment from excised rabbit intestinal mucosa for directly compressed buccal tablets which contained 40 μg of sCT and 0 mg of hakea (□), 12 mg of hakea (◇), or 32 mg of Hakea (○). All data points represent the mean value ± standard deviation of five experiments. Lines through mean values are included to illustrate the trend and do not represent a mathematical fit of the data. The * symbol indicates a statistically significant increase in the mean value of the force of detachment of a tablet containing 12 mg of hakea from that of a tablet containing 0 mg of hakea at $p < 0.05$ using Student's one-tail t-test. The # symbol indicates a statistically significant increase in the mean value of the force of detachment of a tablet containing 32 mg of hakea from that of a tablet containing 12 mg of hakea at $p < 0.05$ using Student's one-tail t-test.

cps) isotonic, hypertonic, and hypotonic formulations, respectively, after intranasal administration of salmon calcitonin at a dose of 2000 IU in rabbits. The percent bioavailability values were 0.14, 0.62, and 0.81 following intranasal administration of high-viscosity (76 cps) formulations prepared with 1% methylcellulose containing 2000 IU of salmon calcitonin in rabbits.[22] Heiber et al.[6] reported the delivery of 550 IU of sCT across canine buccal mucosa but did not report a value for bioavailability. Sinko et al.[18] reported a bioavailability of 16.2 ± 5.1% following subcutaneous (mean value of four different doses) and 0.022 ± 0.018% following intraduodenal administration (mean value of two different doses) in rats. An absolute bioavailability of 1.6% was reported without any permeation enhancer at a dose of 200 IU (40 μg) and 10.3% with 0.5% sodium tauro-24, 25-dihydrofusidate as a permeation enhancer at a dose of 405 IU (90 μg) following intranasal administration of sCT in humans.[23] Estimates of the absolute bioavailability of several other therapeutic polypeptides following buccal administration are 1−5%, 0.1%, and 1% for thyrotropin releasing hormone (TRH) in humans,[24] oxytocin in rabbits,[16] and buserelin in pigs,[17] respectively.

The plasma sCT levels obtained in this investigation using sCT buccal tablets which contained hakea were greater than therapeutic plasma levels (0.1−0.4 ng/mL). These levels were achieved without the use of a permeation enhancer. Heiber et al. also reported sCT plasma levels in excess of the therapeutic range when buccal tablets were evaluated in dogs.[6] The present study demonstrated that the buccal route provided a greater systemic bioavailability than the other routes of sCT administration discussed previously and that the buccal tablets which contained hakea not only achieved therapeutic plasma levels but also sustained the plasma concentration of sCT for 2−3 h. However, in the present study, the authors did observe a departure of plasma sCT concentrations following buccal

administration which would have been anticipated on the basis of our in vitro release studies. More recent findings in our laboratory (unpublished observations) suggest that the increased bioavailability of sCT in the present study may potentially result from hakea inhibiting proteolytic enzymes located in the oral cavity which are responsible for chemical degradation of sCT and/or inhibition of the polypeptide's biological activity. The mechanism of enhanced bioavailability will be the subject of future experimentation.

In the present study, the drug-releasing, uncoated surface adhered to the buccal mucosa during the entire application period. This mucosal binding possibly minimized the loss of drug into the surrounding oral cavity and the gastrointestinal tract in the event that sCT dissolved in saliva were potentially swallowed. Upon the removal of buccal tablets with 40 μg of sCT and 12 mg of hakea, the plasma sCT concentration did not decrease as observed following an intravenous dose suggesting that the sCT may accumulate in the buccal mucosa resulting in a reservoir effect. This has also been observed by Heiber et al.[6]

The pharmacodynamic response to biologically active sCT represents a reduction in plasma calcium concentrations. Figures 3 and 4 illustrate the absorption of biologically active sCT across the buccal mucosa of rabbits. The results demonstrated that the dual compaction process during the manufacturing of the tablet did not affect its biological activity adversely. The values of AAC_{0-2h} for the 40 μg sCT buccal tablets with 12 and 32 mg of hakea were not significantly different ($p > 0.05$), which may be due to the potency of sCT, i.e., the dose required to elicit the maximum pharmacological response is very small. As such, this pharmacological response reaches a maximum beyond which further lowering of plasma calcium concentrations does not occur as the dose is increased (negative feedback control of plasma calcium).[25] Sinko et al.[16] estimated that the maximal lowering of plasma calcium occurs at a plasma sCT concentration of 10 pg/mL in rats. Dua et al.[22] obtained a similar maximal percent decrease in plasma calcium concentrations (% max_d) following intranasal administration of different formulations in rabbits, although the (%) values reported for the bioavailability were quite different. This may have resulted from homeostasis and negative feedback control. Many investigators rely on the pharmacodynamic response to assess the absolute bioavailability[26–29] which may result in an overestimation for the reasons cited above.

Sustained-release, mucoadhesive dosage forms have the advantage of not only adhering to the mucus membrane for the required length of time but also sustaining the release of drug substances. In the present study, the amount of hakea incorporated into the buccal tablet was observed to be a critical factor in defining the resulting bioadhesive strength. A potential reason for an increase in the mucoadhesive bond strength with increasing hakea content (Figure 5) may be due to enhanced water uptake by the gum resulting in tablet swelling and mobilization of flexible polysaccharide chains for interpenetration and physical entanglement with the mucus.

Hakea possesses both hydroxyl and carboxyl terminal groups which can contribute to bioadhesion. Both these functional groups have to be in the un-ionized form in order for it to optimally interact with the negatively charged mucin molecule (under neutral or slightly acidic conditions). The bioadhesive strength increased with time and reached a plateau (Figure 5), suggesting that the process of bioadhesion is saturable and that the mechanism of bioadhesion is likely due to chain interpenetration and physical entanglement[30] of hakea with mucus rather than secondary bond formation (e.g., hydrogen bonding). The fact that the bioadhesive strength reaches a plateau at later time points could be due to the limited surface area of the tablet and exhaustion of the sialic acid residues of the mucin molecule with which the gum can interact in the circular surface area covered by the tablet.

In conclusion, the ability of the novel gum to sustain the release of sCT has been demonstrated both in vitro and in vivo. Moreover, in vitro bioadhesive strength versus time measurements demonstrated that the gum possessed excellent mucoadhesive properties. The tablets were convenient to apply and remove from the buccal mucosa and did not appear to damage the underlying tissue. The mechanism of bioadhesion may potentially result from chain interpenetration and physical entanglement of hakea with the mucus layer. The rate of release of the drug substance as well as the bioadhesive bond strength of the formulation can be modulated by varying the amount of hakea included in the tablet. Thus, the polysaccharide bioadhesive gum hakea may be utilized for not only the sustained delivery of a variety of water-soluble, low molecular weight drug substances but also therapeutic polypeptides.

References and Notes

1. Lang, S.; Rothen-Rutishauser, B.; Perriard, J.; Schmidt, M. C.; Merkle, H. P. Permeation and pathways of human calcitonin (hCT) across excised bovine nasal mucosa. *Peptides* **1998**, *19*, 599−607.
2. Harris, D.; Robinson, J. R. Bioadhesive polymers in peptide drug delivery. *Biomaterials* **1990**, *11*, 652−658.
3. Patel, S.; Lyons, A. R.; Hosking, D. J. *Drugs* **1993**, *46*, 594−617.
4. Hosking, D. J.; Bijvoet, O. L. M. Therapeutic Uses of calcitonin. In *Endocrinology of Calcium Metabolism*; Parsons, J. A., Ed.; Raven Press: New York, 1982; pp 485−535.
5. Lee, K. C.; Soltis, E. E.; Newman, P. S.; Burton, K. W.; Mehta, R. C.; DeLuca, P. P. In vivo assessment of salmon calcitonin sustained release from biodegradable microsphere. *J. Controlled Release* **1991**, *17*, 199−206.
6. Heiber, S. J.; Ebert, C. D.; Dave, S. C.; Smith, K.; Kim, S. W.; Mix, D. In-vivo buccal delivery of calcitonin. *J. Controlled Release* **1994**, *28*, 269−271.
7. Rathbone, M. J.; Drummond, B. K.: Tucker, I. G. The oral cavity as a site for systemic drug delivery. *Adv. Drug Delivery Rev.* **1994**, *13*, 1−22.
8. Merkle, H. P.; Anders, R.; Sandow, J.; Schurr, W. In *Delivery systems for peptide drugs*; Davis, S. S., Lllum, L., Tomlinson, E., Eds.; Plenum Press: New York, 1986; pp 159−175.
9. Ahuja, A.; Khar, R. K.; Ali, J. Mucoadhesive delivery systems. *Drug Dev. Ind. Pharm.* **1997**, *23*, 489−515.
10. Alur, H. H.; Pather, S. I.; Mitra, A. K.; Johnston, T. P. Evaluation of Hakea gibbosa as a sustained-release and mucoadhesive component in buccal tablets. *Pharm. Dev. Technol.* **1999**, *4*, 347−358.
11. Alur, H. H.; Pather, S. I.; Mitra, A. K.; Johnston, T. P. Transmucosal delivery of chlorpheniramine maleate using a novel natural mucoadhesive gum as an excipient of buccal tablets. *Int. J. Pharm.* **1999**, *188* (1), 1−10.
12. Eagles, P. F. K. Structures of complex plant polysaccharides, Exudates from Hakea sericea and Hakea gibbosa. Ph.D. Thesis, Department of Chemistry, University of Capetown, South Africa, March 1992.
13. Peppas, N. A.; Sahalin, J. J. A simple equation for the description of solute release III. Coupling of diffusion and relaxation. *Int. J. Pharm.* **1989**, *57*, 169−172.
14. Gibaldi, M.; Perrier, D. *Pharmacokinetics*, 2nd ed.; Marcel Dekker: New York, 1982; pp 445−449.
15. Hoogstraate, A. J.; Verhoef, J. C.; Pijpers, A.; van Leengoed, L. A. M. G.; Verheijden, J. H. M.; Junginger, H. E.; Bodde, H. E. In vivo buccal delivery of the peptide drug buserelin with glycodeoxycholate as an absorption enhcncer in pigs. *Pharm. Res.* **1996**, *13*, 1233−1237.
16. Li, C.; Bhatt, P. P.; Johnston, T. P. Transmucosal delivery of oxytocin to rabbits using a mucoadhesive buccal patch. *Pharm. Dev. Technol.* **1997**, *2*, 265−274.
17. Kobayashi, S.; Kondo, S.; Juni, K. Study on pulmonary delivery of salmon calcitonin in rats: Effects of protease inhibitors and absorption enhancers. *Pharm. Res.* **1994**, *11*, 1239−1243.
18. Sinko, P. J.; Smith, C. L.; Mcwhorter, L. T. M.; Stern, W.; Wagner, E.; Gilligen, J. P. Utility of pharmacodynamic

measures for assessing the oral bioavailability of peptides. I. Administration of recombinant salmon calcitonin in rats. *J. Pharm. Sci.* **1995**, *84*, 1374−1378.
19. Batra, V.; Bhowmick, A.; Behera, B. K.; Ray, A. R. Sustained-release of ferrous sulfate from polymer-coated gum arabica pellets. *J. Pharm. Sci.* **1994**, *83*, 632−635.
20. Banga, A. K.; Chien, Y. W. Hydrogel-based iontotherapeutic delivery devices for transdermal delivery of peptide/protein drugs. *Pharm. Res.* **1993**, *10*, 697−702.
21. Beveridge, T.; Niederer, W.; Nuesch, E.; Petrin, A. Z. *Z. Gastroenterol.* **1979**, *236*, E15−E19.
22. Dua, R.; Zia, H.; Needham, T. The influence of tonicity and viscosity on the intranasal absorption of salmon calcitonin in rabbits. *Int. J. Pharm.* **1997**, *147*, 233−242.
23. Lee, W. A.; Ennis, R. D.; Longenecker, J. P.; Bengtsson, P. The bioavailability of intranasal salmon calcitonin in healthy volunteers with and without a permeation enhancer. *Pharm. Res.* **1994**, *11*, 747−750.
24. Schurr, W.; Knoll, B.; Ziegler, R.; Anders, R.; Merkle, H. P. Comparative study of intravenous, nasal, oral and buccal TRH administration among healthy subjects. *J. Endocrinol. Invest.* **1985**, *8*, 41−45.
25. Mohamadi, M.; Becker, K. L.; Bivins, L. E. Paradoxical effects of salmon calcitonin on serum calcium: Studies on intact and thyroparathroidectomized men and dogs. *Acta Endocrinol.* **1975**, *79*, 351−355.
26. Kobayashi, S.; Kondo, S.; Juni, K. Pulmonary delivery of salmon calcitonin dry powders containing absorption enhancers in rats. *Pharm. Res.* **1996**, *13*, 80−83.
27. Santi, P.; Volpato, N. M.; Bettini, R.; Catellani, P. L.; Massimo, G.; Colombo, P. Transdermal iontophoresis of salmon calcitonin can reproduce the hypocalcemic effects of intravenous administration. *Farmaco* **1997**, *52*, 445−448.
28. Schipper, N. G.; Verhoef, J. C.; Romeijn, S. G.; Merkus, F. W. Methylated beta-cyclodextrins are able to improve the nasal absorption of salmon calcitonin. *Calcif. Tissue Int.* **1995**, *56*, 280−282.
29. Golomb, G.; Avramoff, A.; Hoffman, A. A new route of drug administration: intrauterine delivery of insulin and calcitonin. *Pharm. Res.* **1993**, *10*, 828−833.
30. Park, H.; Robinson, J. R. Physico-chemical properties of water insoluble polymers important to mucin/epithelial adhesion. *J. Controlled Release* **1985**, *2*, 47−57.

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