

Review

# Transmucosal delivery systems for calcitonin: a review

Madeline Torres-Lugo, Nikolaos A. Peppas\*

*Biomaterials and Drug Delivery Laboratories, School of Chemical Engineering, Purdue University, West Lafayette, IN 47906-1283, USA*

Received 6 April 1999; accepted 16 December 1999

## Abstract

The commercial availability of peptides and proteins and their advantages as therapeutic agents have been the basis for tremendous efforts in designing delivery systems for such agents. The protection of these agents from biological fluids and physiological interactions is crucial for the treatment efficacy. One such agent is salmon calcitonin, a 32 amino-acid polypeptide hormone used in the treatment of bone diseases such as Paget's disease, hypercalcemia and osteoporosis. Researchers have studied different routes to deliver salmon calcitonin more effectively, including nasal, oral, vaginal and rectal delivery. These systems are designed to protect the polypeptide from the biological barriers that each delivery route imposes. Oil-based and polymer-based delivery systems are discussed. © 2000 Elsevier Science Ltd. All rights reserved.

*Keywords:* Calcitonin; Drug delivery; Biodegradable polymers; Non-biodegradable polymers; Hydrogels

## Contents

1. Introduction . . . . .	1191
2. Biochemistry of calcitonins . . . . .	1191
3. Controlled delivery systems for calcitonin . . . . .	1192
3.1. Drug delivery of CT using biodegradable polymers . . . . .	1192
3.2. Oral CT delivery systems using non-biodegradable polymers . . . . .	1194
4. Conclusions . . . . .	1195
Acknowledgements . . . . .	1195
References . . . . .	1195

## 1. Introduction

For many years, the lack of industrial manufacturing processes for peptides and proteins had limited their use as therapeutic agents. However, in recent years the biotechnology and genetic engineering fields have advanced

dramatically, making possible the availability of numerous such therapeutic agents for clinical use [1].

Unfortunately, proteins possess characteristics such as low bioavailability and chemical stability problems [2] that may limit their use for treatment of certain diseases. The delivery of peptides and proteins to the body is usually performed by frequent injections. This results in a rapid increase and subsequent rapid decrease of the blood serum concentration levels that could lead to the appearance of side effects. Therefore, the major challenge in this field is to design a system capable of maintaining a blood concentration for a considerable amount of time inside the therapeutic region and to reduce the number of doses that have to be administered. However, this is not always necessary. For example, in the case of diabetes, 'feedback' delivery of insulin is preferred over constant release. To this date, very few protein delivery systems have been commercialized due to the complexity in the development of a generalized delivery system for peptides and proteins.

This review focuses specifically on the work that has been conducted in the controlled release of calcitonins from polymeric matrices and oil-based formulations.

## 2. Biochemistry of calcitonins

Calcitonin (CT) is a polypeptide hormone comprised of 32 amino acids. It is secreted by the C cells in the

\* Corresponding author. Tel.: 1-765-494-7944; fax: 1-765-494-4080.  
E-mail address: peppas@ecn.purdue.edu (N.A. Peppas).

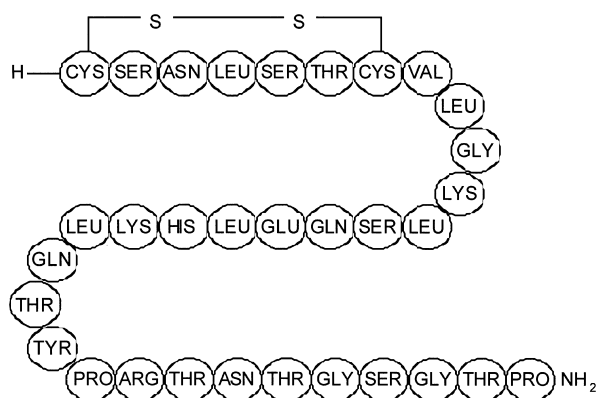


Fig. 1. Amino acid sequence of salmon calcitonin.

thyroid gland and in other vertebrates by the ultimobranchial gland [3]. The basic structure of the CTs is characterized by a disulfide bridge between the cysteine residues at positions 1 and 7 and a proline amide moiety at the C-terminus [4].

The amino-acid sequence of the calcitonins has been determined for many species. Fig. 1 shows the amino-acid sequence for salmon calcitonin (sCT). Specific amino-acid residues are identical for all CTs. Studies [4] of this protein indicate that these similarities seem to be vital for the biological activity of the CTs in other animals rather than the species from which it is naturally produced. Moreover, it has been found that salmon (sCT) and eel (eCT) calcitonins are more potent in mammals, especially in man, than the actual human or other mammalian CTs [5]. The reason for this phenomenon is still not fully understood.

The major physiological role of CT is to control the calcium concentration as well as its metabolism in the body. Its primary responsibility is to reduce the amount of calcium in the blood stream. For this purpose, it increases the rate of calcium clearance from the kidney. It also reduces the amount of calcium excreted by the bone, by inhibiting the osteoclast activity (decreased bone resorption). Finally, it decreases the amount of calcium that could be absorbed from the small intestine. Its production is inhibited when the calcium concentration is decreased beyond normal levels and the parathyroid hormone (PTH) is then secreted. PTH promotes the opposite reactions in the body than CT. In conjunction, these two hormones act to maintain a normal concentration of calcium in the bloodstream.

Calcitonins, due to their ability to reduce osteoclast activity, are commonly used in the treatment of bone diseases such as Paget's disease, hypercalcemia, and osteoporosis. Unfortunately, like other peptides and proteins, CTs are delivered mainly by intramuscular injection, thus, limiting their use as therapeutic agents.

### 3. Controlled delivery systems for calcitonin

Currently, the most common delivery route to administer sCT is through intramuscular or intravenous injections. Various researchers have studied different systems to deliver sCT through various delivery routes (see also Table 1). The most common include nasal, rectal, oral, and vaginal systems as well as implants.

In the case of vaginal and intrauterine systems it has been demonstrated that sCT can be successfully delivered [6–9]. However, this route restricts the type of patients that could benefit from the treatment. It is well known that bone diseases do not affect exclusively women. Men are also in risk of suffering these diseases. Therefore, additional efforts have been put into biodegradable, nasal, and oral systems. The only such system that has successfully reached the market is the nasal formulation for sCT. This formulation is currently used in Europe and has been recently approved for clinical use in the United States by the FDA.

#### 3.1. Drug delivery of CT using biodegradable polymers

Drug delivery systems based on biodegradable polymers are preferred in many biomedical applications because such systems are broken down either by hydrolysis or by enzymatic reaction into non-toxic molecules. The rate of degradation is controlled by manipulating the composition of the biodegradable polymer matrix.

These types of systems are used for the long-term release of therapeutic agents. They are usually designed to act directly in the bloodstream, while protecting the agent from the harmful environment. Biodegradable polymers such as poly(glycolic acid) (PGA), poly(lactic acid) (PLA), and poly(D,L-lactic-co-glycolic acid) (PLGA), have received considerable attention as possible drug delivery carriers, since the degradation products of these polymers have been found to have low toxicity. During the normal metabolic function of the body these polymers degrade into carbon dioxide and water [10]. These polymers have also exhibited excellent biocompatibility.

Lee et al. [11] studied an injectable biodegradable system based on PGA microspheres for the sustained release of sCT. This system was evaluated by sCT release in rats, and its efficiency was compared to that of injectable sCT without any controlled delivery carrier. A sustained hypocalcemic effect (reduced calcium serum concentration) was obtained.

Mehta et al. [10] studied a system of PLGA for the sustained release of sCT. Different preparation parameters for PLGA microspheres were considered and their effect on the *in vivo* release behavior of sCT was studied. PLGA microspheres were prepared by different solvent extraction techniques. sCT was incorporated both during microsphere preparation and after the

Table 1  
Research in the field of controlled drug delivery of calcitonins

Authors	Year	Delivery system	Intended delivery route	Reference
Lee et al.	1991	PGA <sup>a</sup>	Intramuscular	[11]
Mehta et al.	1994	PLGA <sup>b</sup>	Intramuscular	[10]
Jeyanthi et al.	1996	PLGA <sup>b</sup>	Intramuscular	[12]
Calis et al.	1995	PLGA <sup>b</sup>	Intramuscular	[13]
Kawashima et al.	1998	PLGA <sup>b</sup>	Intramuscular	[14]
Brunner et al.	1998	PLA <sup>c</sup>	Intramuscular	[15]
Aydin and Akbuga	1996	Chitosan	Intramuscular	[16]
Pontiroli et al.		Spray	Nasal	[28]
Lowe and Temple	1994	Poly(isobutyl cyanoacrylate)	Oral	[17]
Bai et al.	1996	Carbopol®	Oral	[18]
Baluom et al.	1997	Submicron emulsions containing Carbopol®	Oral, rectal	[20]
Serres et al.	1997	P(NiPAAm-co-BMA-co-AA) <sup>d</sup>	Oral	[21]
Sakuma et al.	1997	P(S-co-MAA) <sup>e</sup> P(S-co-NiPAAm) <sup>f</sup> P(S-co-NVA) <sup>g</sup>	Oral	[22–24]
Torres-Lugo and Peppas	1999	P(MAA-g-EG)	Oral	[25]
New and Kirby	1997	Oil-based formulation	Oral	[27]

<sup>a</sup>Poly(glycolic acid).

<sup>b</sup>Poly(lactic-co-glycolic acid).

<sup>c</sup>Poly(lactic acid).

<sup>d</sup>Poly(*N*-isopropylacrylamide-co-butylmethacrylate-co-acrylic acid).

<sup>e</sup>Poly(styrene-co-methacrylic acid).

<sup>f</sup>Poly(styrene-co-*N*-isopropylacrylamide).

<sup>g</sup>Poly(styrene-co-*N*-vinylacetamide).

formation of the microspheres by adsorption. Adsorbed sCT was found to form multiple layers in the polymer surface. Moreover, it was observed that sCT was capable of binding to the polymer matrix by hydrophobic as well as ionic forces. In vivo release studies indicated that polymer matrices with entrapped sCT were able to induce a hypocalcemic effect for an average of six days, while those with adsorbed sCT had an effect for only four days.

Other researchers studied extensively biodegradable systems based on PGA, PLA, and PLGA, with emphasis on the effects of different solvent removal techniques, polymer preparation techniques, as well as the interactions between sCT and the biodegradable polymer. Jeyanthi et al. [12] studied an aqueous emulsification process to prepare the PLGA microspheres. They investigated different solvent removal techniques in detail and their effect on both the surface structure of the biodegradable PLGA microspheres and the loading efficiency of sCT. The structure of the microspheres depended upon the preparation technique. Microspheres prepared by the temperature gradient method were found to be hollow spheres with porous walls, while the dilution technique produced honeycomb-like structures. The loading efficiency was not affected by the solvent removal technique.

The interactions between the sCT and PLGA microspheres were studied by Calis et al. [13]. PLGA microspheres were loaded with sCT by imbibition. A high-adsorption capacity for sCT was observed.

Formation of multi-layers described by Freundlich isotherms was discovered at higher sCT concentrations.

Although preparation techniques for biodegradable microsphere production have been well studied, the preparation techniques for nanospheres are still under investigation. Kawashima et al. [14] studied two different preparation methods of PLGA nanospheres containing eel calcitonin (eCT). Nanospheres prepared by the emulsion diffusion method in oil showed an increased efficiency of encapsulation of eCT. They were able to release eCT for a period of 14 days compared to a couple of days for those prepared in aqueous solution. This difference was attributed to the particle size. The use of water in the emulsion technique produced nanospheres with an average particle size of 250 nm, whereas the use of oil in the diffusion method produced nanoparticles with a particle size of approximately 700 nm.

A different approach to study the interaction inside biodegradable polymers was reported by Brunner et al. [15]. In this study, atrial natriuretic peptide (ANP) and sCT were labeled with a fluorescent amine-reactive probe, and used to investigate the physical location of the proteins inside the biodegradable polymer matrix. Since the fluorescence intensity was pH-dependent, any changes in pH during the degradation of the polymer matrix could be studied in detail. In this work, PLA microspheres were used and were successfully loaded with the labeled protein. A fluorescent imaging technique was used to physically detect the protein inside the microsphere.

Natural biodegradable polymers have also received attention as possible carriers for peptides and proteins. Aydin and Akbuga [16] reported the use of chitosan, for sCT delivery. They were able to successfully incorporate and release the protein *in vitro* for a period of 27 days using high sCT concentrations. The release profiles of sCT from chitosan microspheres were shown to be non-Fickian in nature.

### 3.2. Oral CT delivery systems using non-biodegradable polymers

Polymeric matrices have been studied extensively as protein carriers in the gastrointestinal tract. A different alternative to achieve this goal can be oil-based formulations.

Lowe and Temple [17] studied nanoparticles composed of two different polymers, polyacrylamide and poly(isobutyl cyanoacrylate) for the oral delivery of human calcitonin (hCT) and insulin. These two systems were examined for their ability to reduce enzyme or proteolytic degradation in the small intestine. In the case of the polyacrylamides no protection from enzymatic attack was observed. However, in the case of poly(isobutyl cyanoacrylate), a small decrease in the enzyme degradation was obtained compared to that of the free peptide. However, both systems seemed to be unsuccessful in protecting the proteins from proteolytic degradation.

Carboxylic acid containing polymers have received considerable attention due to their ability to inhibit proteolytic degradation. Bai et al. [18] studied the ability of different grades of Carbopol® polymers to impede the degradation action of the enzymes trypsin and chymotrypsin on sCT, insulin, and insulin-like growth factor. Carbopol® is the trademark name of a series of polyacrylic polymers designed and produced by BF Goodrich. *In vitro* studies showed that certain types of Carbopols® were able to reduce proteolytic degradation. Lueßen et al. [19] investigated in detail the properties of a grade of Carbopol® as an enzymatic inhibitor. The secondary structure of trypsin was observed to change under the influence of the poly(acrylates). The inhibitory effect was attributed to the capacity of the poly(acrylates) to bind large amounts of cations. The activity of trypsin is affected in the absence of these cations. Baluom et al. [20] studied the absorption of sCT in the jejunum and the colon of rats using submicron emulsions (MA-SME) containing Carbopol® 940. Results showed that diffusion of sCT in a side-by-side diffusion cell using jejunum mucosal epithelium of rat was not enhanced when the submicron emulsions containing Carbopol® 940 were tested. However, the same procedure using colonic mucosal epithelium did show an absorption enhancement compared to sCT in saline solution. The bioavailability of an intracolonic administration of sCT in rats using MA-

SME was found to be 14.7% relative to the same dose administered in saline.

A different polymeric system was studied by Serres et al. [21]. The system consisted of poly(*N*-isopropyl acrylamide-co-butylmethacrylate-co-acrylic acid), a temperature and pH-sensitive hydrogel. The acrylic acid (AA) moiety in these hydrogels gave the system swellability and pH-sensitivity. The percent of AA in the hydrogel was varied and the loading and release efficiency compared. It was observed that polymers with higher AA content (i.e. more hydrophilic) showed the best loading, protein stability, and release efficiency for human calcitonin (hCT). The biological activity of the hCT incorporated into the polymer beads was determined *in vivo*. The loaded hCT was removed from polymer beads and injected into rats. The results showed no difference in the hypocalcemic effect produced by hCT loaded and released from the polymer to that of fresh hCT injected intramuscularly. However, the system was not tested directly in the gastrointestinal tract.

A complete study of different polymeric carriers for sCT was performed by Sakuma et al. [22–24]. Nanoparticles with a hydrophobic backbone composed of polystyrene were copolymerized with hydrophilic grafts of poly(*N*-isopropyl acrylamide) (PNiPAAm), poly(methacrylic acid) (PMAA), poly(*N*-vinylacetamide) (PNVA), and polyvinylamine (PVAm). *In vitro* results showed that the incorporation efficiency was the highest for nanoparticles containing PMAA, regardless of the particle size. This higher incorporation efficiency was due to the electrostatic, hydrogen bonding and hydrophobic interactions between sCT and the polymer chain.

However, results of *in vivo* studies showed that the strongest hypocalcemic effect was obtained from nanoparticles containing PNiPAAm, regardless of the particle size and the macromonomer molecular weight. For all the systems studied, the hypocalcemic effect was sustained for 4 h.

The absorption enhancement of the PNiPAAm nanoparticles was further studied in terms of the effects of dose scheduling *in vivo* [23]. Throughout the experiments, it was found that a dose administered in two different time intervals prolonged the hypocalcemic effect compared to the same dose at a single administration. This phenomenon was thought to be due to the interactions between the nanoparticles and the gastrointestinal mucosa. However, an experimentally proven explanation of these phenomena was not reported.

The next step in this work was the study of the ability of these systems to protect sCT from proteolytic degradation [22]. *In vitro* studies showed that nanospheres containing a polystyrene hydrophobic backbone and hydrophilic grafts were able to protect the protein from enzymatic degradation. The chemical structure of the graft seemed to play an important role in the extent of protection that these systems could give.

Most recently, Torres-Lugo and Peppas studied a different polymeric system composed of crosslinked poly(methacrylic acid) grafted with poly(ethylene glycol) (PMAA-g-EG) gels as a possible oral delivery carrier for salmon calcitonin [25]. *In vitro* studies demonstrated that salmon calcitonin was successfully loaded and released *in vitro*. P(MAA-g-EG) hydrogels were prepared using different amounts of solvent in order to manipulate the three-dimensional structure. The release of sCT was found to be constant for approximately 7 h and was completely released in approximately two days. The rate of release was not very much affected by the amount of solvent used during the polymer preparation.

Another type of oral delivery system consisting of oil-based formulations for hydrophilic peptides and proteins has been recently developed [26,27]. In these formulations, oil is used as the carrier for the peptide through the gastrointestinal tract. This new technology is called Bridgelock™ and was designed and developed by Cortecs Corporation in the United Kingdom. In this technology a water-in-oil emulsion is prepared [27].

In the case of hydrophilic drugs such as sCT, the drug is contained in the aqueous phase. Solid particles are then coated with the emulsion and dried. In the process of drying, the water is removed from the emulsion and the protein is embedded in the oil by using surfactants and stabilizers. In the case of sCT, this method was tested *in vivo* using pigs [26].

Using this technology the method of transport in the gastrointestinal mucosa was shown to be transcellular. The cells of the small intestine have the highest uptake of oil, and therefore, in the presence of the formulation, the membrane permeability is increased leading to the uptake of the oil with the drug. After passing through this barrier the formulation continues its journey into the bloodstream. *In vivo* experiments with pigs were performed by injecting the formulation directly into the jejunum (first section of the small intestine) through a catheter. Results showed a significant hypocalcemic effect when compared to the nasal formulation. Human trials have shown that the efficacy of this system is similar to that of the nasal formulation.

#### 4. Conclusions

Numerous polymer-based delivery systems for calcitonin have been developed. However, the ability of these systems to protect the polypeptide from its environment has not been fully tested in some cases. The low bioavailabilities in those that had been tested *in vivo* demonstrate the difficulty in designing successful delivery systems. Yet, the studies presented here offer a good nucleus of biomaterial-based devices that could be improved for better transmucosal delivery of calcitonin.

#### Acknowledgements

This work was supported in part by grant no. GM43337 from the National Institutes of Health.

#### References

- [1] Swann PW. Recent advances in intestinal macromolecular drug delivery via receptor-mediated transport pathways. *Pharm Res* 1998;16:826–34.
- [2] Putney SD, Burke PA. Improving protein therapeutics with sustained-release formulations. *Nature Biotech* 1998;16:153–7.
- [3] Cholewinski M, Luckel B, Horn H. Degradation pathways. Analytical characterization and formulation strategies of a peptide and protein calcitonin and human growth hormone in comparison. *Pharm Acta Helv* 1996;71:405–19.
- [4] Windich V, De Luccia F, Herman F, et al. Degradation pathways of salmon calcitonin in aqueous solution. *J Pharm Sci* 1997;86:359–64.
- [5] Potts JR. Chemistry of calcitonins. *Bone and Mineral* 1992;16:169–73.
- [6] Golomb G, Avramoff A. A new route of drug administration: intrauterine delivery of insulin and calcitonin. *Pharm Res* 1993;10:828–33.
- [7] Golomb G, Shaked I, Hoffman A. Intrauterine administration of peptide drugs for systemic effect. *Adv Drug Delivery* 1995;17:179–90.
- [8] Richardson JL, Ramírez AP, Miglietta MR, et al. Novel vaginal delivery systems for calcitonin: I. Evaluation of HYAFF/calcitonin microspheres in rats. *Int J Pharm* 1995;115:9–15.
- [9] Richardson JL, Ramírez AP, Miglietta MR, et al. Novel vaginal delivery systems for calcitonin: II. Preparation and characterization of HYAFF microspheres containing calcitonin. *Int J Pharm* 1996;144:19–26.
- [10] Mehta RC, Jeyanthi R, Calis S, et al. Biodegradable microspheres as depot for parenteral delivery of peptide drugs. *J Control Rel* 1994;29:375–84.
- [11] Lee KC, Soltis EE, Newman PS, et al. *In vivo* assessment of salmon calcitonin sustained release from biodegradable microspheres. *J Control Rel* 1991;17:199–206.
- [12] Jeyanthi R, Thanoo BC, Mehta RC, et al. Effect of solvent removal technique on the matrix characteristics of poly(lactic/glycolide) microspheres for peptide delivery. *J Control Rel* 1996;38:235–44.
- [13] Calis S, Jeyanthi R, Tsai T, et al. Adsorption of salmon calcitonin to PLGA microspheres. *Pharm Res* 1995;12:1072–6.
- [14] Kawashima Y, Yamamoto H, Takeuchi H, et al. Properties of a peptide containing DL-lactide/glycolide copolymer nanospheres prepared by novel emulsion solvent diffusion methods. *Eur J Pharm Biopharm* 1998;45:41–8.
- [15] Brunner A, Minamitake Y, Gopferich A. Labelling peptides with fluorescent probes for incorporation into degradable polymers. *Eur J Pharm Biopharm* 1998;45:265–73.
- [16] Aydin Z, Akbuga J. Chitosan beads for the delivery of salmon calcitonin: preparation and release characteristics. *Int J Pharm* 1996;131:101–3.
- [17] Lowe PJ, Temple CS. Calcitonin and insulin in isobutyl cyanoacrylate nanocapsules: protection against proteases and effect on intestinal absorption in rats. *J Pharm Pharmacol* 1994;46:547–52.
- [18] Bai JPF, Chang LL, Guo JH. Effects of poly(acrylic) polymers on the degradation of insulin and peptide drugs by chymotrypsin and trypsin. *J Pharm Pharmacol* 1996;48:17–21.

- [19] Lueßen HL, Verhoef CJ, Borchard G, et al. Mucoadhesive polymers in peroral peptide drug delivery. II. Carbomer and polycarboxophil are potent inhibitors of the intestinal proteolytic enzyme trypsin. *Pharm Res* 1995;12:1293–8.
- [20] Baluom M, Friedman DI, Rubinstein A. Absorption enhancement of calcitonin in the rat intestine by carbopol-containing submicron emulsions. *Int J Pharm* 1997;154:235–43.
- [21] Serres A, Baudys M, Wankim S. Temperature and pH-sensitive polymers for human calcitonin delivery. *Pharm Res* 1996;13:196–201.
- [22] Sakuma S, Ishida Y, Suzuki N, et al. Stabilization of salmon calcitonin by poly(styrene) nanoparticles having surface hydrophilic polymeric chains, against enzymatic degradation. *Int J Pharm* 1997;159:181–9.
- [23] Sakuma S, Suzuki N, Kikuchi H, Hiwatari K, et al. Absorption enhancement of orally administered salmon calcitonin by poly(styrene) nanoparticles having poly(*N*-isopropylacrylamide) branches on their surfaces. *Int J Pharm* 1997;158:69–78.
- [24] Sakuma S, Suzuki N, Kikuchi H, et al. Oral peptide delivery using nanoparticles composed of novel graft copolymers having hydrophobic backbone and hydrophilic branches. *Int J Pharm* 1997;149:93–106.
- [25] Torres-Lugo M, Peppas NA. Novel pH-sensitive hydrogels for the oral delivery of calcitonin. *Macromolecules* 1999;32:6646–51.
- [26] Flynn, M. Oral delivery of insulin and calcitonin in humans. IBC third Annual International Conference of Delivery of Peptides and Proteins. Coronado, California, 1997.
- [27] New RRC, Kirby CJ. Solubilization of hydrophilic drugs in oily formulation. *Adv Drug Delivery Rev* 1997;25:59–69.
- [28] Pontiroli AE. Peptide hormones: review of current and emerging uses by nasal delivery. *Adv Drug Delivery Rev* 1998;29:81–7.