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Novel vaginal delivery systems for calcitonin: I. Evaluation of HYAFF/calcitonin microspheres in rats

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Abstract

The vaginal absorption of salmon calcitonin (sCT) from a solution and after incorporation into microspheres of hyaluronane esters (HYAFF) was studied in rats. Hypocalcaemic responses were markedly enhanced by the use of HYAFF microspheres. Microscopic examination showed good adhesion of microspheres to the vaginal epithelium. The performance of various pessary bases for the vaginal administration of HYAFF and sCT microspheres was evaluated. Vaginal pessaries based on Suppocire BS_2X were the most suitable of those tested, maintaining the bioadhesion of HYAFF microspheres and absorption of sCT. Changes in plasma calcium levels were similar after vaginal administration of this formulation (100 IU/kg sCT) and s.c. injection of sCT solution (10 IU/kg).

Keywords: Intravaginal absorption; Calcitonin; Hyaluronic acid; Microsphere

1. Introduction

Salmon calcitonin (sCT) is a polypeptide hormone which is known to inhibit osteoclastic activity (Singer et al., 1976) and to play a role in calcium homeostasis by inhibiting bone resorption, increasing urinary excretion of calcium and inhibiting the absorption of calcium in the gastrointestinal tract (Cooper et al., 1978; Deftos, 1978). Consequently, sCT is used in the treatment of postmenopausal osteoporosis and Paget's disease of the bone and is employed in the management of malignant hypercalcaemia (Carstens and Feinblatt, 1991). In common with other therapeutic peptide and protein drugs, sCT is normally administered parenterally due to its low bioavailability after oral administration. However, both nasal and rectal routes have been shown to be effective for the delivery of sCT (Buclin et al., 1987) and nasal sCT preparations are now commercially available.

More recently, the potential of the vaginal route for the administration of sCT has been evaluated (Richardson et al., 1992a). The vaginal

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epithelium is permeable to a wide range of drugs, including peptides and proteins, and with its substantial surface area and rich blood supply, the vagina provides a promising site for systemic drug delivery. In addition, prolonged contact of a delivery system with the vaginal mucosa may be achieved more easily than at other absorptive sites, such as the nasal cavity and rectum. Factors affecting the vaginal absorption of drugs, such as age and the menstrual cycle, have been reviewed by Richardson and Illum (1992). Importantly, changes in vaginal histology are minimised after menopause. Indeed, the reduced epithelial thickness in postmenopausal women may facilitate enhanced vaginal absorption of drugs (Furuhjelm et al., 1980).

The vaginal absorption of a model peptide, insulin, has been investigated in rats and in sheep (Richardson et al., 1992b,c). In both models, vaginal absorption of insulin from a simple solution was low but was markedly increased by the coadministration of surfactants. Studies in rats demonstrated that the absorption enhancement was often accompanied by unwanted and at times severe, histological changes in the vaginal epithelium (Richardson et al., 1992b). In the sheep model, the effect of bioadhesive starch microspheres on the vaginal absorption of insulin was evaluated (Richardson et al., 1992c). Reductions in plasma glucose levels were greater and more prolonged in animals treated with the insulin and starch microspheres.

In the present studies, HYAFF microspheres, based on chemically modified hyaluronic acid (HA), have been investigated as novel delivery systems for the vaginal administration of sCT. HYAFF microspheres have previously been evaluated as delivery devices for nerve growth factors (Ghezzo et al., 1992) and for the nasal delivery of insulin (Illum et al., 1994). The preparation and characterisation of HYAFF/sCT microspheres is reported in a separate paper (Rochira et al., in preparation).

The vaginal absorption of sCT was compared after administration of HYAFF/sCT microspheres and sCT solution to rats. In addition, sCT solution was administered by intravenous (i.v.) and subcutaneous (s.c.) routes. Due to the difficulties associated with the assay of plasma sCT concentrations (Pagani et al., 1991; Di Perri et al., 1992), plasma calcium levels were measured after administration of the sCT formulations. A reduction in plasma calcium levels provides a measurement of calcitonin absorption and has been used as an index of osteoclastic activity (Alexandre et al., 1979; Blanc et al., 1977).

As in previous vaginal absorption studies in the rat, the animals were ovariectomised and treated with oestradiol to provide a model displaying consistent vaginal histology (Richardson et al., 1992b). The efficacy of the microspheres was also determined after incorporation in various pessary bases, in order to develop practical and convenient vaginal delivery systems.

2. Materials and methods

2.1. Materials

sCT was obtained from Bachem Fine Chemicals (Torrance, U.S.A.). HYAFF 11 and HYAFF 11p75 were supplied by Fidia S.p.A. (Abano Terme, Italy). All materials used in the preparation of microspheres are described elsewhere (Rochira et al., in preparation). 17-β-Oestradiol, sesame oil and corn oil were purchased from Sigma Chemical Co. (St. Louis, MO). Colloidal silica, Aresoli 200 was obtained from Eigenmann Veronelli (Italy). Suppocire BM and BS_2X were a gift from Gattefossé (Milan, Italy).

2.2. Preparation of sCT solutions

sCT solutions for vaginal and parenteral administration, respectively, were prepared in saline adjusted to pH 4 with 0.1 N HCl and in normal saline (pH 5.7). All other sCT solutions were prepared in distilled water.

2.3. Preparation of HYAFF / sCT microspheres

Microspheres of HYAFF 11 and HYAFF 11p75 containing sCT (5 IU/mg) were prepared as described elsewhere (Rochira et al., in preparation).

Table 1
Details of treatment groups and doses of sCT administered

Formulation	Dose of sCT (IU/kg)
Vaginal sCT solution	100
Vaginal HYAFF 11/sCT powder	100
Vaginal HYAFF 11p75/sCT powder	100
Vaginal pessaries (oil/silica) containing HYAFF 11/sCT	100
Vaginal pessaries (Suppocire BM) containing HYAFF 11/sCT	100
Vaginal pessaries (Suppocire BS_2X) containing HYAFF 11/sCT	100
Intravenous sCT solution	5
Subcutaneous sCT solution	10

2.4. Preparation of pessary formulations

HYAFF 11/sCT microspheres (5 mg) were dispersed in pessaries (100 μl) based on Suppocire BM or Suppocire BS_2X. In addition, microspheres (25 mg/ml) were suspended in corn oil containing silica at a concentration of 40 mg/ml.

2.5. Absorption studies

Female Wistar rats (Charles-River, Como, Italy) weighing approx. 200 g were used. The animals were ovariectomised and treated with oestradiol, as described previously (Richardson et al., 1992b). Groups of rats ($n = 4$) were anaesthetised by intraperitoneal injection of pentobarbitone sodium, 60 mg/kg. After tracheotomy and cannulation of the carotid artery and jugular vein, sCT formulations were administered by vaginal, s.c. and i.v. routes. Details of the formulations used and doses administered are shown in Table 1. Liquid vaginal formulations were administered as before (Richardson et al., 1992b) while pessaries were inserted with the aid of a moistened cotton tampon. Dry microsphere formulations were loaded into polypropylene capillary tubes (i.d. 1.7 mm, o.d. 2.7 mm) and injected into the vagina using a positive displacement micropipette. sCT injected intravenously was administered via the jugular cannula.

Blood samples were withdrawn from the arterial cannula and were collected in heparinised tubes prior to drug administration and at intervals over 6 h. Plasma was separated by centrifugation and stored at −20°C awaiting analysis. Plasma calcium concentration was determined by atomic absorption spectrometry (BM Hitachi 737 System) and expressed as a percentage of the basal concentration.

At the end of the absorption experiments, rats were killed by an overdose of pentobarbitone sodium. In some groups, the vaginas were removed and processed for histology.

Table 2
Mean maximum fall in plasma calcium concentrations (as percent change from basal concentration) and mean time of maximum fall, calculated from individual rat data

Formulation	Mean maximum fall (C_{max}) (%) of basal ± SE)	Time of maximal fall (T_{max}) (min ± SE)
Vaginal sCT solution, 100 IU/kg	11.6 ± 1.31	195 ± 15
Vaginal HYAFF 11/sCT powder, 100 IU/kg	24.1 ± 1.81	135 ± 15
Vaginal HYAFF 11p75/sCT powder, 100 IU/kg	20.3 ± 2.64	120 ± 34.6
Vaginal pessaries (oil/silica) containing HYAFF 11/sCT, 100 IU/kg	11.2 ± 2.10	172.5 ± 55.3
Vaginal pessaries (Suppocire BM) containing HYAFF 11 sCT, 100 IU/kk	7.7 ± 1.35	270 ± 57.4
Vaginal pessaries (Suppocire BS_2X) containing HYAFF 11 sCT, 100 IU/kk	20.2 ± 1.8	252 ± 35
Intravenous sCT solution, 5 IU/kg	29.4 ± 1.2	285 ± 15
Subcutaneous sCT solution, 10 IU/kg	23.2 ± 1.67	264 ± 24

3. Results

3.1. Effect of HYAFF microspheres

Vaginal administration of HYAFF 11/sCT and HYAFF 11p75/sCT microspheres resulted in more pronounced hypocalcaemic effects than a simple sCT solution (Fig. 1). Mean maximal decreases in plasma calcium concentrations were 24 and 20% in rats treated with HYAFF 11/sCT and HYAFF 11p75/sCT microspheres, respectively, compared to 12% in animals treated with sCT solution (Table 2). Furthermore, maximal hypocalcaemic effects occurred more rapidly after administration of microsphere formulations (T_{max} 120 and 135 min) than after administration of sCT solution (T_{max} 195 min).

Microscopic examination of the rat vaginal epithelium after administration of both HYAFF 11/sCT and HYAFF 11p75/sCT clearly showed the presence of numerous microspheres in the vaginal lumen and closely attached to the vaginal epithelium (Fig. 3b). The histological effects of the formulations were slight with some crenation of the surface cell layer in areas in contact with the microspheres. The thickness and histology of the vaginal epithelium did not differ greatly from that of control animals (Fig. 3a).

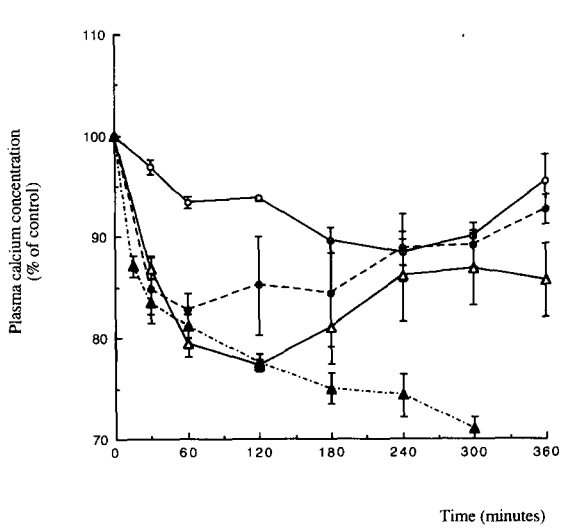

Fig. 1. Mean changes in plasma calcium concentrations (%) after vaginal administration of sCT (100 IU/kg) solution and dry microsphere formulations and i.v. injection of sCT solution (5 IU/kg) to rats ($n = 4$); (○) vaginal sCT solution, (△) HYAFF 11/sCT, (●) HYAFF 11p75/sCT, (▲) i.v. sCT solution.

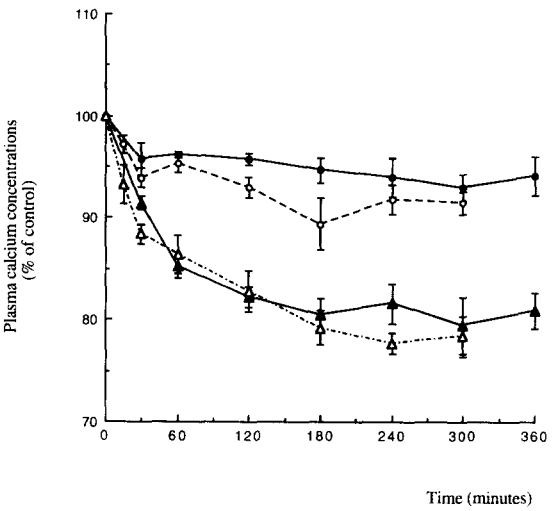

Fig. 2. Mean changes in plasma calcium concentrations (%) after vaginal administration of pessaries containing HYAFF 11/sCT (100 IU/kg) and s.c. injection of sCT solution (10 IU/kg) to rats ($n = 4$); (○) corn oil/silica, (●) Suppocire BM, (▲) Suppocire BS_2X, (△) s.c. sCT solution.

3.2. Effect of pessary formulations

Plasma calcium concentrations following the vaginal administration of pessary formulations containing HYAFF 11/sCT microspheres are shown in Fig. 2. Only slight decreases in plasma calcium levels (approx. 8% of basal, Table 2) occurred after vaginal administration of microspheres incorporated in Suppocire BM pessaries. Similarly, only slight hypocalcaemic effects were attained after administration of microspheres suspended in oil and silica (mean maximal decrease 11% of basal levels, Table 2). In contrast, after vaginal administration of Suppocire BS_2X pessaries containing HYAFF 11/sCT, a marked decrease in plasma calcium concentrations of approximately 20% of basal concentrations was observed after 4 h (Table 2).

Macroscopic observation of the vagina following administration of Suppocire BM formulation revealed the presence of partially melted pessary base. These specimens were not examined under

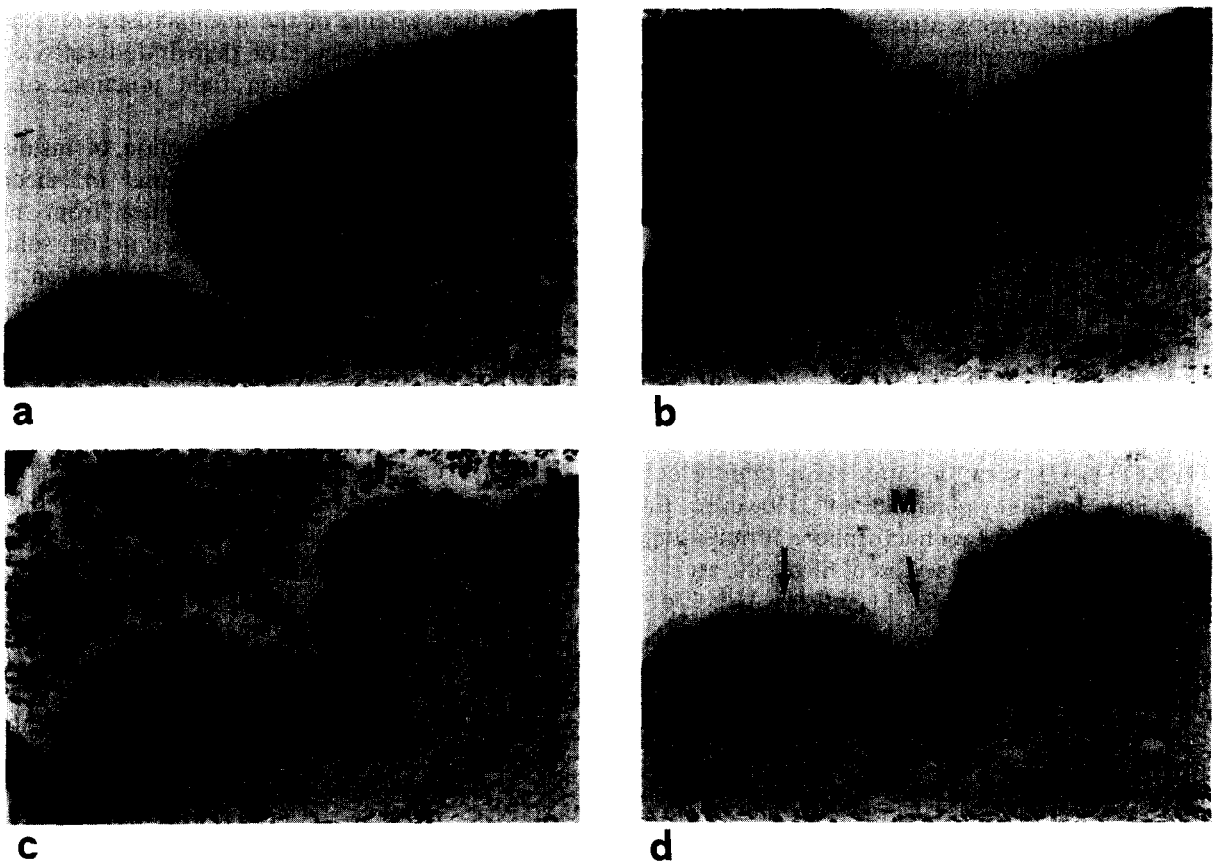

Fig. 3. Photomicrographs of sections of vaginal tissues of rats from control group and groups treated with microsphere formulations. Magnification, −200. (a) Control group. The vaginal epithelium (E) comprises five to six cell layers organised in the form typical of a stratified squamous epithelium. (b). HYAFF 11 microspheres (dry powder) group. The epithelium (E) closely resembles that of the control group. Numerous microspheres (M) are evident in the vaginal lumen and in contact with the epithelium (arrows). (c). Suppocire BS_2X pessary group. Microspheres are well dispersed throughout the vaginal lumen (L) and demonstrate adhesion to the epithelial surface (E). No evidence of changes in the thickness and histology of the vaginal epithelium. (d). Corn oil/silica pessary. Film of oil covers epithelial surface (arrows). Few microspheres (M) are visible in vaginal lumen.

the light microscope. After administration of the Suppocire BS_2X pessary, microspheres tended to be less aggregated than when used as a dry powder and showed good attachment to the vaginal mucosa (Fig. 3c). Microspheres suspended in oil and silica showed poor bioadhesion with the oily vehicle evident in the vaginal lumen and on the epithelial surface (Fig. 3d).

3.3. Comparison of vaginal and parenteral administration of sCT

Changes in plasma calcium concentrations were pronounced following i.v. and s.c. injection of 5 and 10 IU/kg of sCT with maximal decreases of 29 and 23% of basal observed after 4 h (Fig. 1 and 2; Table 2). Profiles of plasma calcium concentrations with time were similar after s.c administration of sCT (10 IU/kg) and vaginal administration of HYAFF 11/sCT microspheres (100 IU/kg) in the Suppocire BS_2X pessary (Fig. 2).

4. Discussion

Incorporation of sCT into HYAFF microspheres greatly improved the vaginal absorption of the peptide with more rapid and pronounced

hypocalcaemic effects attained after administration of the microsphere formulations than after administration of sCT solutions. This enhancement of absorption may be due to the intimate contact achieved between the microspheres and the vaginal mucosa, resulting in high local concentrations of the peptide at the site of absorption. Histological examination of the vaginal epithelium 6 h after administration of the dry microspheres revealed that the microspheres were closely attached to the epithelial cells, lying in small concavities of the surface layer. Microspheres of HYAFF 11 tended to show a greater affinity for the vaginal epithelium than those of HYAFF 11p75, with the latter being more widely dispersed in the vaginal lumen. However, the distribution of the two microsphere formulations was not quantitatively assessed. Slight differences in the efficacy of HYAFF 11 and HYAFF 11p75 microspheres were also observed, with greater decreases in plasma calcium occurring in animals treated with HYAFF 11. These differences could be due to the relative bioadhesion of the two polymers after vaginal application or to slight differences in release rates of the peptide from the microspheres. Studies to assess the bioadhesive properties of HYAFF microspheres using in vitro techniques are ongoing. In general, bioadhesive polymers tend to be hydrophilic macromolecules with substituents capable of forming hydrogen bonds, in particular carboxyl groups (Smart et al., 1984). In theory, HYAFF 11p75, in which 75% of carboxyl groups are esterified with benzyl alcohol and 25% are present as the sodium salt, should show greater bioadhesion than HYAFF 11 (total benzyl ester). However, the greater hydrophobic character of HYAFF 11 may encourage its attachment to the vaginal mucosa, minimising its distribution in the aqueous environment of the vaginal lumen.

It has been proposed that bioadhesive microsphere systems may induce transient widening of intercellular junctions when applied nasally (Illum and Davis, 1992). Ryden and Edman (1992) demonstrated that tight junctions in Caco-2 cell monolayers were wider following application of starch microspheres, with a subsequent increase in the uptake of model compounds. They suggested that swelling of the dry microspheres and a consequent shrinkage of dehydrated cells accounted for the effect on tight junctions and increased drug transport.

Evaluation of the nasal absorption of insulin from HYAFF microsphere systems in sheep demonstrated an enhanced absorption from the microspheres compared with a nasal insulin solution (Illum et al., 1994). Nasal administration of insulin solution and insulin/HYAFF microspheres resulted in bioavailabilities of 1.2 and 11% of that of an s.c. injection. The absorption enhancement by the microspheres was not thought to be linked to mucosal damage as no change in nasal histology was observed after repeated administration of microspheres for 10 days in rabbits (unpublished results).

Similarly, the histological effects of HYAFF 11 microspheres after vaginal application were slight with some crenation of the surface cell layer observed. In further studies, the histological effects of HYAFF 11 were investigated after repeated administration for 14 days in normal rats. After repeated daily administration of HYAFF 11 as a dry powder and after incorporation in Suppocire BS_2X pessaries, no differences in vaginal histology were observed between treatment groups and controls (in preparation).

The vaginal absorption of sCT from HYAFF/sCT microspheres was markedly affected by incorporation into different pessary vehicles. The rationale for preparing the pessary formulations was to provide a practical device which maintained the bioadhesion of HYAFF microspheres and the stability of sCT, but which showed good local tolerability without aggravating the vaginal dryness often associated with menopause. The three vehicles studied, which are used in commercial vaginal products, showed varying performances. Absorption of sCT was low after administration of HYAFF/sCT microspheres in the Suppocire BM base, which could be due to incomplete melting of the pessary and hence limited availability of the microspheres. The oil and silica suspension was evident in the vaginal lumen and as a film on the mucosal surface, impeding contact of the microspheres and absorption of sCT.

The Suppocire BS_2X base was the most suitable of those tested providing a solid support for the HYAFF/sCT microspheres and good absorption characteristics. This vehicle is more hydrophilic in character than Suppocire BM, consisting of a mixture of semi-synthetic polyethylene triglycerides which melt at 35–37°C and form a fine emulsion on contact with the aqueous environment of the vagina, thus encouraging the dispersion of microspheres and release of sCT.

In conclusion, HYAFF microspheres clearly showed potential as a novel delivery system for the vaginal administration of sCT, resulting in enhanced hypocalcaemic responses. The mechanism of absorption promotion was thought to be linked to the close attachment of the microspheres to the vaginal epithelium. Further studies are underway to measure plasma sCT concentrations directly after administration of sCT formulations to rats. In addition, the efficacy of HYAFF microsphere formulations for the vaginal delivery of sCT has been evaluated in sheep (in preparation).

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