International Journal of Pharmaceutics 423 (2012) 257–263

Contents lists available at SciVerse ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Delivery of salmon calcitonin using a microneedle patch

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ARTICLE INFO

Article history:
Received 23 August 2011
Received in revised form
16 November 2011
Accepted 28 November 2011
Available online 8 December 2011

Keywords:
Microneedle
Peptide delivery
Salmon calcitonin
Transdermal drug delivery

ABSTRACT

Peptides and polypeptides have important pharmacological properties but only a limited number have been exploited as therapeutics because of problems related to their delivery. Most of these drugs require a parenteral delivery system which introduces the problems of pain, possible infection, and expertise required to carry out an injection. The aim of this study was to develop a transdermal patch containing microneedles (MNs) coated with a peptide drug, salmon calcitonin (sCT), as an alternative to traditional subcutaneous and nasal delivery routes. Quantitative analysis of sCT after coating and drying onto microneedles was performed with a validated HPLC method. In vivo studies were carried out on hairless rats and serum levels of sCT were determined by ELISA. The AUC value of MNs coated with a trehalose-containing formulation (250 ± 83 ng/mL min) was not significantly different as compared to subcutaneous injections (403 ± 253 ng/mL min), but approximately 13 times higher than nasal administration (18.4 ± 14.5 ng/mL min). T_{max} (7.5 ± 5 min) values for MN mediated administration were 50% shorter than subcutaneous injections (15 min), possibly due to rapid sCT dissolution and absorption by dermal capillaries. These results suggest that with further optimization of coating formulations, microneedles may enable administration of sCT and other peptides without the need for hypodermic injections.

1. Introduction

Recent advances in biotechnology have made it possible to use biological macromolecules, such as peptides and proteins, as therapeutic agents. Calcitonin, which is a cyclic polypeptide of 32 amino acids (molecular weight of approximately 3450 Da), has a physiological role in the regulation of calcium homeostasis and is a potent inhibitor of osteoclastic bone resorption (Azria et al., 1995). Calcitonin is found in pigs and humans, and even in the ultimobranchial gland of birds and fish. Salmon calcitonin (sCT) has been used preferentially because of its higher potency compared to other sources as a therapeutic agent to treat postmenopausal osteoporosis, hypercalcemia and symptomatic Paget's disease of bone (Schneyer, 1991).

sCT has been commercialized in the form of intramuscular (IM) and subcutaneous (SC) injections and nasal spray formulations (Torres-Lugo and Peppas, 2000; Physicans' Desk Reference, 2011). The main limitations of current injectable formulations are nausea and facial flushes caused by a high blood concentration peak (Harvey and Withrow, 1985). Furthermore, IM injection has problems such as infection at the site of injection, pain caused by needle insertion and poor patient compliance.

To overcome these limitations, nasal delivery was introduced for simple patient administration. However, current nasal formulations irritate nasal mucosa and cause side effects such as rhinitis, rhinorrhea, and allergic rhinitis (Ugwoke et al., 2001). These side effects have been difficult to eliminate because nasal formulations typically require absorption enhancers that increase transmucosal sCT delivery, but also cause irritation. Even with the use of absorption enhancers, the bioavailability of sCT is much lower than that following IM and SC injection, i.e., only approximately 3% for clinically used sprays (Lee et al., 1994).

To avoid the above problems, transdermal administration has been proposed to take the place of injection and nasal application (Chang et al., 2000). Transdermal drug delivery is especially attractive, because patches offer a simple and painless way to administer drugs. However, the tough barrier posed by the skin's outer layer, stratum corneum, has generally limited the transdermal route to drugs that are hydrophobic, low molecular weight, and potent (Prausnitz and Langer, 2008a), which precludes sCT. Iontophoresis has been shown to increase sCT delivery into the skin, but requires a sophisticated electronic device (Chaturvedula et al., 2005).

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doi:10.1016/j.ijpharm.2011.11.046

Table 1
Composition of MN coating formulations.[a]

Coating formulation code	sCT (w/v %)	CMCNa (low viscosity) (w/v %)	Trehalose (w/v %)	Lutrol F-68 NF (w/v %)
MN1	1	1	–	0.5
MN2	1	1	15	0.5

[a] Coating formulations were prepared with deionized water.

In this study, we propose the use of a microneedle (MN) patch to administer sCT via skin. MN patches use an array of micron-scale needle-like structures to pierce into the superficial layers of the skin in a painless manner (Gill et al., 2008; Prausnitz et al., 2009; Donnelly et al., 2010; Birchall et al., 2011; Sachdeva and Banga, 2011). Drugs or vaccines can be administered in this way either for local effect in the skin or systemic distribution via capillary uptake.

Four different types of microneedle designs have been developed, which include solid microneedles that pierce the skin to make it more permeable, solid microneedles coated with dry powder drugs for dissolution in the skin, microneedles prepared from polymers with encapsulated drugs for rapid or controlled release in the skin, and hollow microneedles for injections (Prausnitz et al., 2008b).

We have chosen to use solid MNs coated with a dry-powder drug formulation that dissolves off the MNs upon insertion in the skin (Gill and Prausnitz, 2007). Previous studies have used this approach to administer other peptides, including desmopressin in preclinical studies (Cormier et al., 2004) and parathyroid hormone in clinical trials (Daddona et al., 2011), as well as other compounds, notably including influenza vaccine (Zhu et al., 2009; Kim et al., 2010; Fernando et al., 2010) and other vaccines (Andrianov et al., 2009; Prow et al., 2010; Hiraishi et al., 2011). We believe this is the first study to report on sCT delivery using MNs.

2. Materials and methods

2.1. Materials

sCT was purchased from Calbiochem (San Diego, CA, USA) for MN coatings, Novartis Pharma Stein (Miacalcin Injection, East Hanover, NJ, USA) for SC and intravenous (IV) injections, and Par Pharmaceutical Companies (Calcitonin-Salmon Nasal Spray USP, NY, USA) for intranasal (IN) instillation. We also used carboxymethylcellulose sodium salt (CMCNa, low viscosity, USP grade, Carbo-Mer, San Diego, CA, USA), Lutrol F-68 NF (BASF, Mt. Olive, NJ, USA), and D-(+)-trehalose dihydrate (Sigma–Aldrich, St. Louis, MO, USA) for MN coatings. Active® Ultra-Sensitive sCT ELISA kit was purchased from Diagnostic Systems Labs (Webster, TX, USA).

2.2. Methods

2.2.1. MN fabrication

MNs were fabricated from stainless steel sheets (SS 304, 50 μm thick, Trinity Brand Industries, Atlanta, GA, USA) as five-needle arrays, each MN measuring 730 μm long, 180 μm wide at the base, 50 μm in thickness, and tapering to a sharp tip with less than 3 μm radius of curvature, as described previously (Gill and Prausnitz, 2007). MN were coated using two different coating solution formulations, as shown in Table 1. MN coating was performed by dipping each MN eight times into the coating solution at 25 °C using a specially designed apparatus with computer-controlled linear stages (Newmark Systems, Rancho Santa Margarita, CA, USA) and a video camera (Prosilica, Newburyport, MA, USA) to monitor the process.

2.2.2. In vitro assay of sCT on coated MNs

Four MN arrays, each containing five coated MNs, were incubated in 1 mL deionized water for 5 min to completely dissolve sCT. The solution was filtered through a membrane filter having a pore diameter of 0.45 μm and analyzed with a validated HPLC method (Tas et al., 2012). Briefly, chromatographic separation was performed using a reverse-phase Agilent Eclipse XDB-C18 column (150 × 4.6 mm i.d., 3.5 μm particle size, Greensboro, NC, USA). The mobile phase consisted of acetonitrile and water (35:65 v/v) containing 0.1% trifluoroacetic acid degassed prior to use. The column temperature was 65 °C and the flow rate was set at 1 mL/min. Triamcinolone acetonide was used as an internal standard. The validation parameters are presented in Table 2.

2.2.3. In vitro dissolution kinetics of sCT from MNs

One array of MNs coated with sCT was dipped into DI water (250 μL) for different periods of time (2, 5, 8, 12 and 20 s). Then, the dissolution medium was filtered and sCT assay was performed by HPLC, as described above.

2.2.4. In vivo bioavailability of sCT in hairless rats

All animal studies were conducted with approval by the Georgia Institute of Technology Institutional Animal Care and Use Committee (IACUC). Twenty-eight hairless male Sprague–Dawley rats (Charles River Laboratories, Wilmington, MA, USA) weighing 300–350 g were equally divided into seven groups (i.e., four rats per group) and fasted for 18 h prior to the experiment, but allowed free access to water. The rats were anesthetized with isoflurane during sCT administration and until the end of the experiment. Although anesthesia may alter sCT pharmacokinetics relative to conscious animals, anesthesia was used to immobilize animals during the study and was used on all animals in all study groups. Blood samples were collected from the tail vein at 0, 5, 15, 30, 45, 60, 90, 120, 180 and 240 min after MN, SC and IN administration and at 0, 5, 10, 15, 30, 45, 60, 90, 120, 180 and 240 min after IV administration. All samples were centrifuged at 10,000 rpm for 10 min and serum was collected (Chaturvedula et al., 2005).

Group 1 received an IV injection of commercial sCT for injection as a positive control. Group 2 received a SC injection of commercial sCT for injection as a positive control. Group 3 received an IN spray of commercial sCT for nasal delivery as a positive control. Groups 4 and 5 received sCT from a MNs patch formulated without or with trehalose, respectively. Groups 6 and 7 received a SC injection of sCT dissolved in DI water from MNs formulated without or with trehalose, respectively.

2.2.4.1. Conventional routes of sCT administration. sCT was administered IV at a dose of 3 μg (i.e., corresponding to approximately 10 μg/kg) given through the femoral vein. sCT was administered SC at a dose of 3 μg at the dorsal hind portion of the rat. For IN administration, rats were placed in the supine position. A dose of

Table 2
Validation parameters of sCT in HPLC analysis.

Linearity range	2.5–50 μg/mL
Correlation coefficient	0.999
Detection limit	1.00 μg/mL
Quantification limit	2.5 μg/mL
Intra-day precision (RSD %)	0.99
Inter-day precision (RSD %)	1.73

RSD: relative standard deviation.