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Delivery of salmon calcitonin using a microneedle patch

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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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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.

5.68 μL per rat (equal to 3 μg) was instilled into the left nares using a pipetter with a disposable plastic tip. After 2 min, the rats were turned over and remained in the prone position.

2.2.4.2. sCT administration using MNs. Two arrays of MNs (coated with a dose equal to approximately 3 μg) were applied to the dorsal section of the rat by gentle pushing. MN were left in the skin for 20 min to be sure that essentially all of the sCT dissolved in tissue fluids. After removal from the skin, we kept the MNs refrigerated until subsequent analysis to determine if any sCT remained on the MNs.

2.2.5. In vivo assay of sCT in serum

Serum samples obtained by the experiments described above were analyzed for sCT concentration using an enzymatically amplified two-step sandwich type immunoassay for salmon calcitonin, validated for rat serum. Briefly, specially coated plates are incubated with the peptide antibody. The unbiotinylated peptide (standards and experimental samples) is then added and allowed to incubate with the antibody to facilitate binding. The biotinylated peptide is then added to the wells which competes for the antibody binding sites with the unbiotinylated peptide. Finally, a biotin-streptavidin detection system is employed to quantify the amount of bound biotinylated peptide which in turn indicates the amount of salmon calcitonin in standards and experimental samples. Standard curves were constructed in the range of 250–4000 pg/mL in rat serum.

2.2.6. Data analysis and statistical evaluation

Results are presented as the mean of $n = 4$ determinations with its associated standard deviation (S.D.). Noncompartmental pharmacokinetic analysis was carried out using the Pharmacologic Calculation System (version 4.1, Springer–Verlag, Philadelphia, PA, USA) computer program which calculates the AUC (area under the curve) of the serum concentration as a function of time. The maximum serum concentration (C_{max}) and the time to reach the maximum serum concentration (T_{max}) were obtained from the experimental data. Absolute (F_{abs}; versus IV) and relative (F_{rel}; versus SC) bioavailability were calculated according to following equations:

$$F_{abs} = \frac{AUC_i/Dose_i}{AUC_{IV}/Dose_{IV}} \times 100\% \quad (1)$$

$$F_{rel} = \frac{AUC_i/Dose_i}{AUC_{SC}/Dose_{SC}} \times 100\% \quad (2)$$

where the subscript i corresponds to SC, IN or MN.

All the results are expressed as means ± S.D. Statistical differences between values were determined using SPSS 15.0 for Windows software (IBM, İstanbul, Turkey), with Student's t-test. The difference was regarded statistically significant when $p < 0.05$.

3. Results

The development of effective formulations for patient-friendly peptide delivery represents a significant challenge in modern pharmaceutical technology. At present, most of these biotherapeutics have to be administered via parenteral routes by injection, which are inconvenient because of pain, fear and risks associated with this type of application (Dorkoosh et al., 2001; Deacon and Abramowitz, 2006; Nir et al., 2003). A more acceptable route of administration with potentially good bioavailability could be offered by transdermal delivery. Recent advances show that coated MN patches can be used to administer peptide drugs to obtain systemic effects (Cormier et al., 2004; Daddona et al., 2011). In this study, we have investigated delivery of sCT with coated MNs and compared the bioavailability of sCT with other drug delivery routes, i.e., subcutaneous and nasal.

3.1. MN fabrication and coating with sCT

MNs fabricated by laser-cutting stainless steel sheets were designed to be long enough to penetrate through the stratum corneum and viable epidermis and into the superficial dermis by gentle manual insertion, but short enough to avoid pain (Gill et al., 2008). Our delivery strategy involved coating these solid MNs with formulations of sCT that dissolve upon insertion into skin. We developed aqueous coating formulations including a surfactant (Lutrol F-68 NF) to facilitate uniform coatings by reducing surface tension, a viscosity enhancer (CMCNa) to enable thicker coatings by increasing coating solution residence time on the MN during the drying process and a sugar derivative (Trehalose) to protect sCT against loss of activity, especially during drying of the coating. Microscopic examination revealed that dip-coating in this way produced uniform coatings localized to MN shafts (Fig. 1).

After dissolution of the coatings and analysis by HPLC, the amount of sCT on MNs was found to be 317 ± 49 and 294 ± 36 ng per needle for coating formulations MN1 and MN2, respectively. In this way, a dose of 3 μg could be administered using two five-needle arrays with a total of 10 MNs. The HPLC method used enabled a relatively rapid analysis (7 min) that saved time and reduced organic solvent use. Because it was an isocratic method, this assay can be done using a simple HPLC instrument.

3.2. Kinetics of in vitro dissolution of sCT from coated MNs

The dissolution of sCT from MNs required only 20 s when incubated in DI water (Fig. 2), although dissolution kinetics in the skin may be slower. There was no significant difference between the dissolution kinetics for MN1 and MN2 formulations. These rapid kinetics are probably due to the coating layer on MNs being very thin and including highly water-soluble excipients that were selected in part to enable rapid dissolution.

3.3. In vivo bioavailability of sCT

3.3.1. IV and SC injection

As positive control experiments, we first injected sCT at a dose of 3 μg (10 μg/kg) via IV and SC routes in hairless rats. The average serum sCT concentration vs. time profile is shown in Fig. 3A. The average peak concentration, C_{max}, was 10.5 ± 7.0 ng/mL and 8.0 ± 2.9 ng/mL and the average area under the curve, AUC, was 389 ± 131 ng mL/min and 403 ± 253 ng mL/min for IV and SC injection, respectively (Table 3). There were no significant differences between C_{max} and AUC values after IV versus SC injection ($p > 0.05$). The average time until peak concentration, T_{max}, was 5 min after IV injection, which was significantly faster than after SC injection, which was 15 min ($p < 0.01$). Because the first data point after IV injection was taken at 5 min, it is possible that the T_{max} is even shorter and the C_{max} is even higher than reported for IV injection.

3.3.2. Nasal administration

The IN route represents an attractive alternative to parenteral delivery for an increasing number of therapeutic peptides. The pharmacokinetic profile obtained after IN administration of a commercial sCT formulation is shown in Fig. 3A. The C_{max} and AUC values were significantly lower compared to IV and SC injections ($p < 0.05$), with an absolute bioavailability of only 4.7 ± 3.7% by the IN route (Table 3). The T_{max} value after IN delivery was not significantly different than the IV and SC routes ($p > 0.05$). This result is consistent with previous findings that absolute bioavailability of sCT after IN administration is around 3% (Sweetman, 2002). Low

Table 3
Pharmacokinetic parameters of sCT after application via different delivery routes in hairless rats ($n = 4$).

Delivery route[a]	Formulation	AUC (ng/mL min)	C_{max} (ng/mL)	T_{max} (min)	F_{abs} (%)[b]	F_{rel} (%)[c]
IV	Commercial (IV)	389 ± 131	10.5 ± 7.0	5	–	–
SC	Commercial (IV)	403 ± 253	8.0 ± 2.9	15	104 ± 65	–
IN	Commercial (IN)	18.4 ± 14.5	0.38 ± 0.47	61 ± 53	4.7 ± 3.7	4.6 ± 3.6
Skin	MN1	154 ± 79	3.8 ± 3.2	15	39.6 ± 20.2	38.2 ± 19.5
Skin	MN2	250 ± 83	6.5 ± 2.6	7.5 ± 5.0	64.3 ± 21.5	62.0 ± 20.7
SC	MN1	267 ± 181	2.0 ± 0.9	30 ± 15	68.7 ± 46.6	66.3 ± 45.0
SC	MN2	229 ± 131	2.3 ± 0.1	45 ± 15	58.9 ± 33.8	56.8 ± 32.6

[a] IV: intravenous; SC: subcutaneous; IN: intranasal.
[b] F_{abs}: absolute bioavailability (relative to IV injection).
[c] F_{rel}: relative bioavailability (relative to SC injection).

bioavailability of sCT via the IN route can be explained by the low membrane permeability and high metabolic turnover of this peptide in the nasal epithelium and a short local residence time of the formulation at the site of absorption (Bernkop-Schnürch et al., 2004).

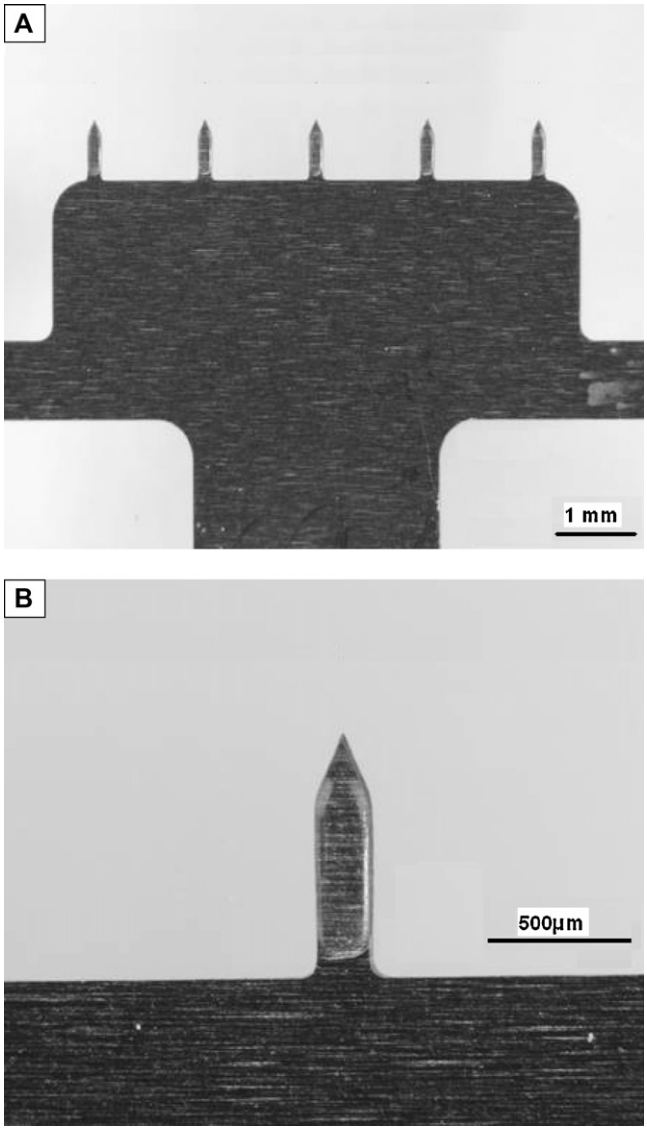

Fig. 1. Microscopic images of MNs coated with sCT. (A) Low-magnification view of a five-needle array of MNs. (B) Higher-magnification view of a single MN. In both images, the stainless steel MNs are coated with a dried formulation containing sCT, as well as a surfactant (Lutrol F-68 NF), a viscosity enhancer (CMCNa) and a peptide stabilizer (trehalose). The MNs each measure 730 μm long, 180 μm wide at the base, 50 μm in thickness, and tapering to a sharp tip with 3 μm radius of curvature.

Prior work has shown that degradation of sCT in excised bovine nasal mucosa was primarily due to enzymatic breakdown by α-chymotryptic and tryptic activity, which could be inhibited by cyclodextrins, resulting with high bioavailability (Sigurjonsdottir et al., 1999). sCT also has a relatively short residence time in the nose, which limits its absorption. The lack of a suitable mucoadhesive polymer in commercial formulations is another possible reason for low bioavailability of sCT via the nasal route (Tas et al., 2009).

3.3.3. *Delivery using MNs*

The pharmacokinetics of sCT delivery using MN is shown in Fig. 3B. Delivery using the MN1 formulation showed a significantly lower C_{max} and higher T_{max} compared to IV injection ($p < 0.05$) and a decreased AUC compared to both IV and SC injection ($p < 0.05$) (Table 3). Compared to IN administration, delivery using the MN1 formulation had a significantly faster T_{max} and larger AUC ($p < 0.05$). This shows that delivery with a MN patch using the MN1 formulation offers improved bioavailability compared to IN delivery, but has a bioavailability significantly smaller than the injection route.

The MN1 formulation contained CMCNa and surfactant, but no trehalose. Addition of trehalose in the MN2 formulation improved

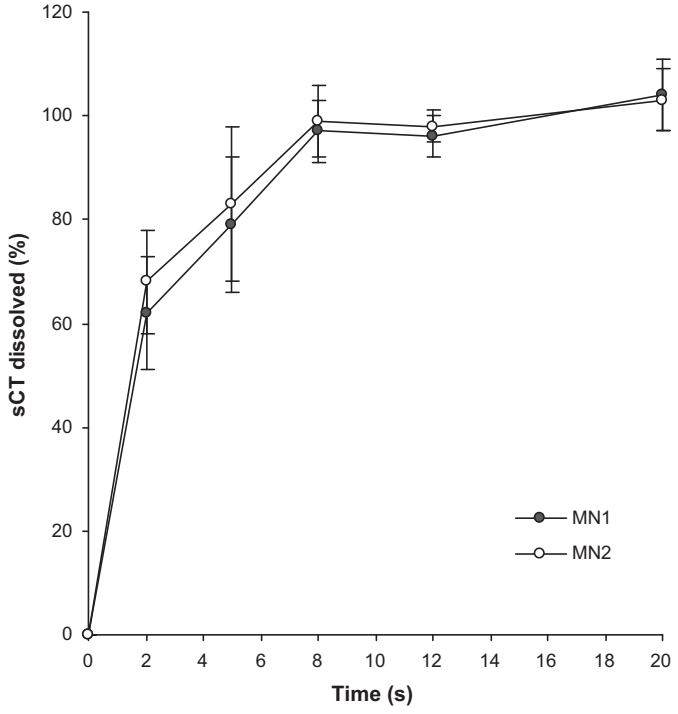

Fig. 2. Dissolution of sCT from coated MNs in DI water. MNs coated using formulation MN1 or MN2 (see Table 1) were incubated in DI water at room temperature to determine dissolution kinetics. Data points represent the average of 4 replicates. Error bars represent the standard deviation.

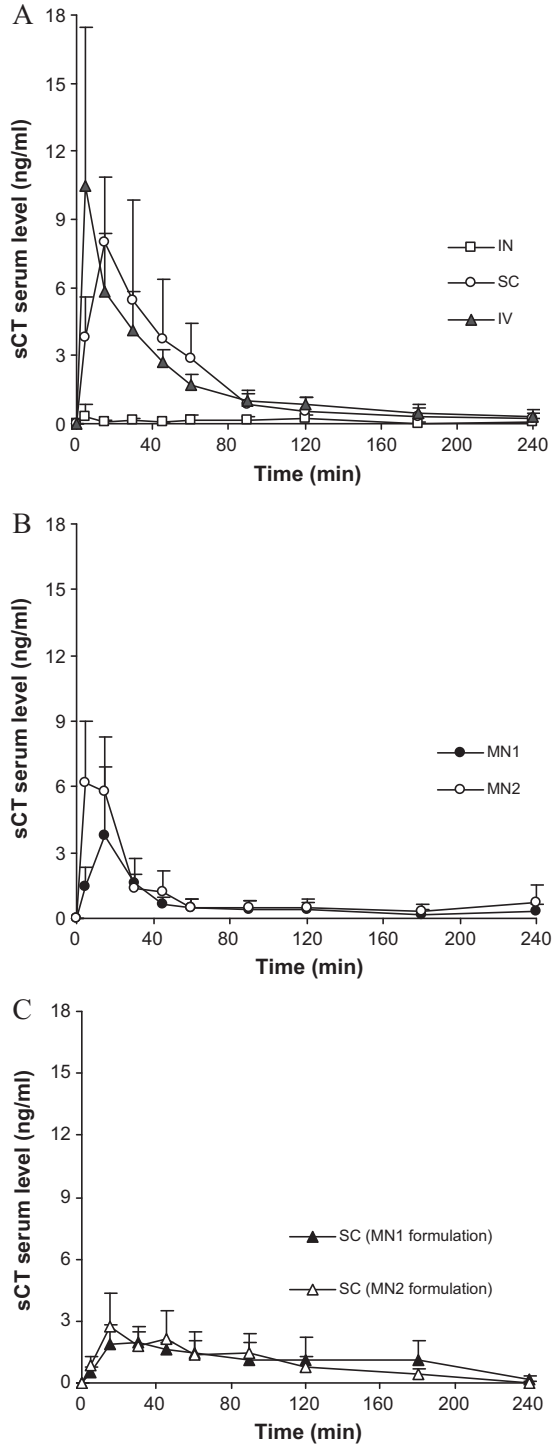

Fig. 3. Serum levels of sCT after administration by different delivery routes with different dosage forms in hairless rats. Pharmacokinetic profiles are shown for (A) IV, SC and IN routes, (B) delivery using MNs with the MN1 and MN2 formulation and (C) SC injection using sCT coated onto MNs using the MN1 and MN2 formulations, and then redissolved in DI water before injection as a liquid formulation. Data points represent the average of 4 replicates, each from a different rat. Error bars represent the standard deviation.

sCT delivery profile (Fig. 3B). For the MN2 formulation, there were no significant differences in C_{max}, T_{max} or AUC compared to IV injection ($p > 0.05$) (Table 3). The C_{max} and AUC values associated with the MN2 formulation were also not significantly different from SC injection ($p > 0.05$). Compared to IN delivery, MNs with the MN2 formulation had significantly faster T_{max} and larger AUC values ($p < 0.05$). This shows that delivery using a MN patch containing trehalose incorporated MN2 formulation had pharmacokinetic parameters that were similar to the injection route, and a dramatic improvement over IN administration.

Although the AUC values for IV injection and MN delivery using the MN2 formulation were statistically indistinguishable, the absolute bioavailability of MN2 delivery was only $64.3 \pm 21.5\%$, while the absolute bioavailability of MN1 delivery was only $39.6 \pm 20.2\%$. We hypothesized that these reduced bioavailabilities might be due to incomplete dissolution of sCT from MNs in the skin and/or from damage to sCT during the MN coating process.

To assess whether incomplete dissolution of sCT might have played a role, we incubated MNs in water after removing from the skin. Subsequent analysis by ELISA found that there was no significant sCT remaining on the MNs after removal from the skin (data not shown). Although we did not measure if sCT might have flaked off or otherwise became deposited on the skin surface during MN insertion, previous studies using similar MN formulations have shown that this probably did not occur (Gill and Prausnitz, 2007). Thus, we conclude that most of the full dose of sCT coated onto the MN was delivered into the skin.

We next considered possible damage to sCT during the coating process. This process involves applying a liquid formulation to the MN surface, which is then air dried to leave a solid film. Substantial drying occurs within 10 s (as seen through visual observation, data not shown), although complete drying occurs over a longer time scale.

To separate the effects of MN fabrication from the MN route of delivery, coated MNs with sCT using the MN1 and MN2 formulation, dissolved in deionised water and then injected the resulting solution by the SC route (Fig. 3C). In this scenario, SC injection of the MN2 formulation had a significantly longer T_{max} compared to SC injection of the commercial formulation. In addition, SC injection of both MN1 and MN2 formulations had significantly longer T_{max} values compared to delivery via MNs ($p < 0.05$) (Table 3). SC injections of MN coating formulations also had significantly smaller C_{max} values as compared to SC injection of the commercial formulation and MNs mediated delivery ($p < 0.05$). Despite these differences, the AUC values for SC injections of either of the MN formulations were not significantly different from SC injection of the commercial formulation and not significantly different from delivery using a MN patch with either formulation ($p > 0.05$).

Altogether, these data suggest that the MN formulation generally slowed the dissolution rate when administered by SC injection, as shown by longer T_{max} and smaller C_{max} values, but nonetheless yielded similar AUC values. Although the differences in AUC values were not significant, absolute bioavailability of sCT by SC injection using the MN1 and MN2 formulations, was just $68.7 \pm 46.6\%$ and $58.9 \pm 33.8\%$, respectively, which is similar to the values for the MN route of administration (Table 3). Based on the similar bioavailability values seen for MN1 and MN2 formulations when given SC or using a MN patch, we propose that the observed low bioavailabilities can be explained in part by damage to sCT during formulation and MN coating, and may not be related to this route of administration.

4. Discussion

4.1. Bioavailability

Delivery of drugs via coated MNs might benefit from traditional delivery routes such as oral, parenteral and nasal. Like many proteins and peptides, the therapeutic use of calcitonin is restricted due to its physical instability. It is well-known that calcitonin is unstable upon incubation in aqueous solution. pH and

temperature have a dominant effect on aggregation of calcitonin (Mansoor et al., 2005). In aqueous solutions, calcitonin has a pronounced tendency to aggregate into long, thin fibrillar aggregates, yielding a viscous and turbid dispersion (Bauer et al., 1995). A calcitonin solution stored for 20 h at room temperature also indicated the early onset of aggregation (Dijkhuizen-Radersma et al., 2002). Commercial injectable dosage forms of sCT must be formulated with suitable excipients to protect the drug in the formulation and in the biologic environment (Lee et al., 1992).

Our coating formulation MN1 contained only CMCNa and a surfactant. In the MN2 formulation trehalose was also added to study its effect as a stabilizing agent, especially during coating and drying processes. There were no other excipients such as pH buffer or enzyme inhibitors, or environmental controls such as low temperature, that could have conferred additional stability to sCT.

It is also known that chemical stability of peptides and proteins in the dry state is enhanced by the presence of certain amorphous sugars (Okamoto et al., 2002). Sugars can protect peptides and proteins against loss of activity through chemical and thermal denaturation. Trehalose has been known to be a superior stabilizer in providing protection to biological materials against dehydration and desiccation (Kaushik and Bhat, 2003). Two hypotheses have been proposed for the mechanism of protein stabilization by an amorphous sugar. The water substitution hypothesis supposes that sugar molecules form hydrogen bonds with dried proteins in place of water molecules to maintain higher-order protein structure. The glassy state theory supposes that the high viscosity of an amorphous sugar prevents proteins from degrading physically or chemically by retarding molecular movement. Thus, the presence of trehalose in the MN2 formulation may play a role in stabilization of sCT.

4.2. Kinetics of drug onset

The T_{max} value of sCT delivery using MN with the MN1 formulation was similar to SC injection ($p > 0.05$) (Table 3). However the T_{max} value for a MN patch using the MN2 formulation was significantly shorter ($p < 0.05$). This can be explained by the thin film of dried sCT on the MNs composed largely of highly water-soluble trehalose in the MN2 formulation that dissolves quickly in skin (Lammert et al., 1998) (Fig. 2). Moreover, skin has been shown to be a site for rapid absorption of drugs into the systemic circulation in previous MN studies (Harvey et al., 2011; Gupta et al., 2011). Surfactant (Lutrol F68, NF) used in both coating formulations also may play a role in the fast dissolution of sCT in skin, because surfactant can increase drug wettability and dispersibility by decreasing surface tension between the drug and the dissolution medium (Prasanthi et al., 2011).

In contrast, SC application of solutions obtained by dissolution of MNs in deionised water, lengthened the T_{max} value ($p < 0.05$). These findings can be explained with the dissolution of coated layer of MNs in deionised water can form a viscous solution due to the presence of CMCNa. Generally polymers used in formulations can cause decrease of the drug release from the formulations and also can extend the drug release period. Also relatively long distances in the SC space to capillaries can be evaluated as another factor (Barichello et al., 1999; Tas et al., 2006).

4.3. sCT metabolism is tissue

Skin is known to contain both exo- and endopeptidases, with aminopeptidases being the best known (Banga and Chien, 1993). For this reason, the viable layers of skin may metabolise drugs. For example, vasopressin was found to degrade during skin transport (Banga et al., 1995; Barry, 2001) and Raiman and co-workers showed that LHRH was degraded in the viable epidermis (Raiman et al., 2004). Recently, several reports introduced the use of proteolytic enzyme-inhibitors to stabilize proteins and peptides during their transmucosal delivery. Mingda and co-workers showed that bestatin (aminopeptidase inhibitor), aprotinin (trypsin inhibitor) and leupeptin (trypsin and cathepsin B inhibitor) had an inhibiting effect on the degradation of [Arg^8]-vasopressin by the skin (Bi and Singh, 2000). The results indicated that the bioavailability of these drugs was significantly improved in the presence of enzyme inhibitors. These results suggest that MN coating formulations containing enzyme inhibitors could increase the bioavailability of sCT.

4.4. Potential therapeutic significance

This study shows that delivery of sCT using a MN patch may offer an alternative to hypodermic injections, with comparatively high bioavailabilities. This could be significant because injections require patient training, are painful, generate sharps waste and, for these reasons and others, lead to decreased patient compliance. A MN patch, in contrast, can be designed to be painless, is readily accepted by patients and healthcare providers, and can be used with little or no training (Gill et al., 2008; Birchall et al., 2011). Although MN production will require new manufacturing methods, mass-produced MN patches are expected to have a cost similar to needle-and-syringe delivery (Prausnitz et al., 2008b). Given the small size of microneedles, delivered doses may be limited to submilligram, but this is sufficient for many peptide and protein drugs. We therefore conclude that sCT delivery using a MN patch may provide an important advance to simplify administration of sCT and other peptide and protein drugs and thereby increase patient access and compliance.

5. Conclusion

Peptide- and protein-based therapies are currently limited by their need for hypodermic injection by needle and syringe. The only current alternative to injections of sCT is intranasal delivery, but this method has extremely low bioavailability issues. In this study, we introduced the use of a microneedle patch to administer sCT without the need for hypodermic needles and found that the bioavailability was not significantly different from conventional subcutaneous injection. This suggests that delivery using a microneedle patch may provide a viable alternative to injection of sCT and other peptide and protein therapeutics.

Conflict of interest

Mark Prausnitz serves as a consultant to companies, is a founding share-holder of companies and is an inventor on patents licensed to companies developing microneedle-based products. This potential conflict of interest has been disclosed and is being managed by Georgia Tech and Emory University.

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