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In vivo iontophoretic delivery and pharmacokinetics of salmon calcitonin

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Abstract

In vivo iontophoretic delivery of salmon calcitonin (SCT) in hairless rats using a self-contained wearable and disposable iontophoretic patch was investigated. Iontophoretic patches with built-in proprietary Zn/AgCl electrodes were used. SCT was formulated in citrate buffer (50 mM, pH 4.0) to impart a positive charge for anodal iontophoresis. SCT was delivered intravenously to determine primary pharmacokinetic parameters. Pharmacokinetics of iontophoretic delivery of SCT was compared with subcutaneous route of administration. Blood samples were collected through tail vein and analyzed for serum SCT and calcium levels. Pharmacokinetic parameters were calculated by non-compartmental analysis. An average current of 0.43 ± 0.01 mA was maintained during patch application. Iontophoretic patches delivered SCT at an average infusion rate of 177.9 ± 58.7 ng/(min kg) and an average steady state concentration of 7.58 ± 1.35 ng/ml was achieved. There was no difference between the calcium lowering effect of iontophoretic patch and subcutaneous injection ($p > 0.05$). Clearance and half-life of SCT after IV administration were found to be 16.8 ± 0.9 ml/(min kg) and 33.5 ± 3.3 min, respectively. The iontophoretic delivery of SCT was well defined by a one-compartment model with zero-order infusion. Iontophoretic patch delivered therapeutically relevant concentrations of SCT in hairless rats and delivery was comparable to conventional routes.

Keywords: Salmon calcitonin; Iontophoresis; Pharmacokinetics; Calcaemia

1. Introduction

Transdermal iontophoresis is one alternative to invasive routes of drug administration for charged macromolecules. Iontophoresis utilizes a constant current source connected to the electrodes in the patch, which makes the currently available units bulky and reduces

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patient compliance. Recently, a wearable electronic device has been made available for localized drug delivery with built-in electrodes in the patch, reducing the size and set-up of the patch and making it more patient-friendly. The present study utilizes this iontophoretic patch and is a first step to establish it as a potential delivery system for systemic delivery of therapeutic peptides. Iontophoresis utilizes a small amount of current to push charged drug molecules through and across the skin and into the systemic circulation. Advantages of this route include improved patient compliance, avoidance of first pass hepatic metabolism, controlled delivery and the possibility to modulate the rate of delivery (Banga, 1998; Banga et al., 1999; Chang et al., 2000).

In recent years, several peptides and proteins have been made available for therapeutic use. However, multiple injections are typically needed, and they usually have a low bioavailability and chemical stability problems which limits their use. Furthermore, these injections may result in large spikes in blood concentration with associated various side effects. Thus, one challenge for delivery of peptides and proteins is to maintain effective non-toxic levels of the drug in blood for prolonged periods of time (Banga and Chien, 1993).

In this study, salmon calcitonin (SCT) was used as model peptide. Calcitonin is a polypeptide hormone consisting of 32 amino acids, secreted by thyroid gland. The primary structure of salmon calcitonin is characterized by a disulfide bridge between the cysteine residues at positions 1 and 7 and a proline amide moiety at the C-terminus. SCT is more potent than human or any other mammalian calcitonins. The major physiological role of calcitonin is control of calcium concentration and metabolism in the body in conjunction with parathyroid hormone. SCT primarily acts by inhibiting osteoclastic bone resorption and by stimulating osteoclastic bone formation. Clinically, SCT is used to treat hypercalcemia, Paget's disease and postmenopausal osteoporosis, and is generally given as subcutaneous or intramuscular injections, or through the nasal route (Torres-Lugo and Peppas, 2000; Zaidi et al., 2002). However, due to a short half-life, multiple injections are required for the optimal pharmacological effect, and thus patient compliance is low. There have been reports in the literature for delivering SCT orally by different strategies such as reversible lipidization (Wang et al., 2003) or PEGylation (Na et al., 2004). Oral route of SCT administration suffers with low bioavailability due to extensive proteolytic degradation. Hee et al. (2000) reported the bioavailability of calcitonin following intraduodenal administration as 0.022% and from 0.2–0.9% after intracolonic administration. Transmucosal delivery systems showed promising results for alternative delivery of calcitonin resulting in a marketed nasal spray formulation of calcitonin with a higher bioavailability than oral route (Torres-Lugo and Peppas, 2000).

Nasal delivery of calcitonin is viable and commercially available but it suffers the disadvantages of irritation of nasal mucosa and variable absorption in case of nasal disease conditions. Major side effects of nasal drug delivery route include ear, nose and throat disorders, such as rhinitis, rhinorrhea or hydrorhea and allergic rhinitis but are of minor intensity.

The main aim of present study is to evaluate the in vivo iontophoretic delivery of SCT using a self-contained iontophoretic patch with built-in electrodes to investigate the pharmacokinetic parameters such as rate of delivery and to compare the pharmacokinetic profiles and calcium lowering effect of iontophoretically delivered SCT with subcutaneous and intravenous injection of SCT.

2. Materials and methods

2.1. Materials

SCT was purchased from Calbiochem (San Diego, CA, USA), citric acid and sodium citrate were purchased from Sigma (St. Louis, MO, USA), Water for Injection (WFI) was purchased from Abbott Laboratories (Chicago, IL, USA), Active® Ultra-Sensitive Salmon Calcitonin ELISA kit was purchased from DSL, Inc. (Webster, TX, USA) and Wako Calcium measurement kit from Wako Chemicals GmbH (Germany).

2.2. Methods

2.2.1. Animals

Male CD-hairless rats (Charles River, Wilmington, MA, USA) weighing 250–350 g were used. The research adhered to the "Principles of Laboratory Animal Care" (NIH publication #85-23, revised in 1985). Food and water were provided ad lib. The average number of replicates for each study was four.

2.2.2. Iontophoretic patch—principle of operation

The Wearable Electronic Disposable Drug delivery (WEDD®) devices were obtained from Birch Point Medical, Inc., and utilized electrodes embedded in absorbent pads (1 cm^2) on medical adhesive tapes. For the electrodes, specific amounts of Zn and AgCl were coated on the anode and cathode, respectively, and these electrodes were connected through an electrically conductive material. Because of the reduction potential difference between Zn and Ag metals an approximately 1 V potential is maintained in the patch when the electrode pads are filled with electrolyte solution which makes the patch self-contained and different from conventional power sources in iontophoretic delivery.

The electrochemistry involved at the electrode interfaces were:

$$Zn \rightarrow Zn^{++} + 2e^{-} (\text{at anode})$$

$$AgCl + e^{-} \rightarrow Ag + Cl^{-} (\text{at cathode})$$

In this investigation, we utilized a WEDD® iontophoretic system consisting of components that can be integrated into self-powered, single-use disposable patches. A first implementation of WEDD® patches is now commercially available (as IontoPatch), and is generally used to deliver anti-inflammatory medications with a 1-V integrated power source over a 24-h period. With WEDD® technology, customized power sources are constructed, with rate of medication delivery adjusted by applied voltage, and duration of delivery adjusted by the material content of the consumable electrodes. In the present study, we established a goal of achieving a rapid delivery profile as close as possible to subcutaneous injection, which through in vitro modeling required the use of a total 10-V power system. To achieve 10 V, the Zn and AgCl WEDD® electrodes (which produce 1 V, as described previously) were placed in series with an additional 9 V source. The electrical requirements for the additional 9 V source were to provide the current and total charge capacity required for the study. Three series-connected 3 V lithium batteries (Panasonic BR 1225, with capacity in excess of 30 mA h) were used to provide the additional voltage. Alternatively, several Zn/AgCl electrodes can be used in series as previously done for delivery of propranolol (Chaturvedula et al., 2003). A 10 kΩ resistance was also placed in series with the power sources to minimize the variations in current flow due to variability in the skin resistance between animals.

2.2.3. In vivo iontophoretic delivery of SCT

Rats were anesthetized using intraperitoneal injection of ketamine (75 mg/kg) and xylazine (10 mg/kg). Once deep anesthesia was induced, the abdominal area of the rat was washed, dried, cleaned with an alcohol swab and finally air-dried. SCT (1 mg/ml) in citrate buffer (50 mM, pH 4.0) was used as drug formulation. SCT (p*I* 10.4) was positively charged at the formulation pH and was thus delivered under the positively charged electrode (anode). The iontophoretic patch, filled with 250 μl of drug formulation in the anode and 0.9% sodium chloride solution in the cathode, was applied onto the cleaned abdominal area and the additional batteries and resistor were connected in series. Patches were applied for 2 h. Current flow was monitored using a multimeter throughout the patch application period. Blood samples were collected through tail vein at 0, 15, 30, 45, 60, 90, 120, 150 and 180 min. Blood samples were allowed to clot and centrifuged at 7200 × *g* for 10 min and serum was collected and stored at −20 °C until analyzed for serum SCT and calcium concentrations.

2.2.4. Intravenous injection (IV) of SCT

Male CD-hairless rats were anesthetized as described before. SCT was dissolved in WFI and 10 μg/kg dose was given intravenously through femoral vein and blood samples were collected at 0, 5, 10, 15, 30, 45, 60, 90, 120 and 180 min through tail vein and treated as described before.

2.2.5. Subcutaneous injection (SC) of SCT

Male CD-hairless rats were anesthetized as described before. SCT was dissolved in WFI and 10 μg/kg dose was given subcutaneously at the dorsal hind portion of the rat. Blood samples were collected at 0, 15, 30, 45, 60, 90 and 120 min through tail vein and treated as described before.

2.3. Assay methods

Serum samples obtained by the experiments described above were analyzed for SCT concentration

using an ELISA kit (DSL Inc., Webster, TX, USA) validated for rat serum. The method is an enzymatically amplified two-step sandwich type immunoassay involving biotin–streptavidin detection system. Standard curves were constructed in the range of 250–4000 pg/ml in rat serum. Serum calcium was measured using a assay kit (Wako Chemicals GmbH, Germany) based on complexation with o-cresopthalein complexon, in alkaline medium, and absorbance was measured at 575 nm.

2.4. Pharmacokinetic data analysis

Serum concentration versus time profiles from IV, SC and iontophoretic routes were analyzed using non-compartmental analysis using WinNonlin (4.1). Pharmacokinetic parameters such as AUC_{0-inf}, terminal elimination rate constant (λ_z), clearance/F, C_{max}, were calculated. Clearance obtained from IV data, was used to calculate the dose delivered during iontophoresis by the following equation assuming that iontophoretic delivery is a zero order infusion:

$$F \times \text{dose delivered} = \text{AUC}_{\text{iontophoretic}} \times \text{clearance}_{\text{IV}} \quad (1)$$

Rate of infusion (R_0) at steady state was calculated by following equation:

$$R_0 = \frac{F \times \text{dose delivered}}{\text{duration of patch application}} \quad (2)$$

where F represents the fraction of dose absorbed into systemic circulation. It represents the drug loss in the skin and subdermal layers. $F \times$ dose delivered will be calculated as a single function from Eq. (1).

Pharmacodynamic response was characterized by area above the effect curve calculated by trapezoidal rule from the time versus % serum calcium profiles.

2.5. Pharmacokinetic modeling

To validate the calculations involved in non-compartmental analysis, the serum profiles were fitted using WinNonlin (4.1) software, to the one-compartmental continuous infusion model with zero order absorption as follows:

$$C_p = \frac{F \times \text{dose delivered}}{\text{Cl}} \times (1 - e^{-kt}) \quad \text{If } t \le T_{inf}$$

$$C_p = \frac{F \times \text{dose delivered}}{\text{Cl}} \times (1 - e^{-kt}) \times (e^{-k(t-T_{inf})}) \quad \text{If } t > T_{inf}$$

where C_p is the serum concentration of SCT, k the elimination rate constant, Cl the clearance and T_{inf} is the time of patch application

The parameter estimation was done using Gauss–Newton algorithm with Levenberg–Hartley modification. A number of other pharmacokinetic models were also evaluated and include the one-compartment model with first-order input and two-compartment models with constant and first-order inputs. Goodness of fit criteria included the Akaike information criteria (AIC) (Ludden et al., 1994), lack of systemic deviations in the residuals and a high correlation coefficient.

2.6. Statistical analysis

All data are presented as mean ± S.E. Statistical analysis was performed using analysis of variance (ANOVA). $p < 0.05$ was regarded as significant.

3. Results and discussion

WEDD® produced and maintained an average current of 0.43 ± 0.01 mA during patch application (Fig. 1). WEDD® delivered SCT at an average infusion

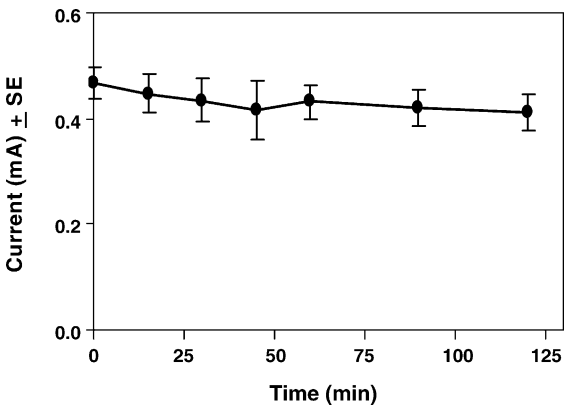

Fig. 1. Current flow during WEDD® application in hairless rats.

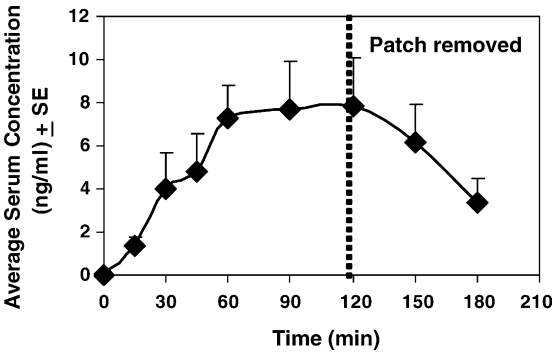

Fig. 2. Serum concentration of SCT after WEDD® application in hairless rats.

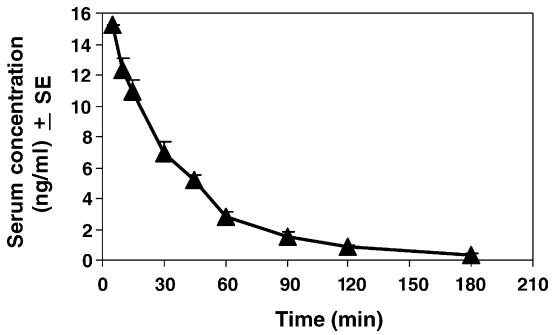

Fig. 3. Serum concentration of SCT after IV administration in hairless rats.

rate of 177.9 ± 58.7 ng/(min kg) and an average steady state concentration of 7.58 ± 1.35 ng/ml was achieved (Fig. 2). The temporal profile of serum SCT concentrations after intravenous injection is shown in Fig. 3 and pharmacokinetic parameters calculated are given in Table 1. Subcutaneous injection of SCT resulted in a C_{max} of 3.12 ± 0.26 ng/ml (Fig. 4). Pharmacokinetic parameters of SCT after SC and iontophoretic routes of administrations are given in Table 2. Serum calcium

Table 1
Pharmacokinetic parameters (average ± S.E.) after IV administration of SCT in hairless rats

Parameter	Value
C_{max} (ng/ml)	15.2 ± 0.7
Cl (ml/(min kg))	16.8 ± 0.9
λ_z (min^{-1})	0.02
Half-life (min)	33.5 ± 3.3
AUC_{inf} (min ng/ml)	603.1 ± 34.0

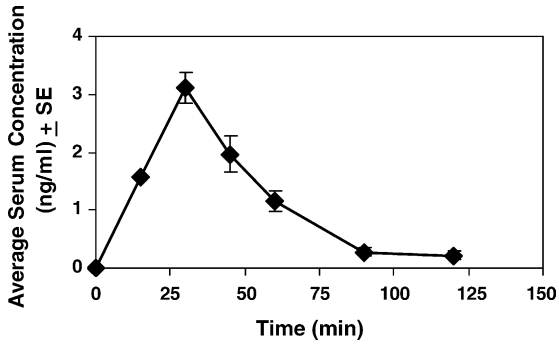

Fig. 4. Serum concentration of SCT after subcutaneous injection in hairless rats.

Table 2
Pharmacokinetic parameters (average ± S.E.) after iontophoretic and subcutaneous injection routes in hairless rats

Parameter	WEDD®	SC injection
C_{max} (ng/ml)	10.1 ± 1.5	3.1 ± 0.2
λ_z (min^{-1})	0.03	0.03
Half-life (min)	35.3 ± 12.9	21.4 + 3.0
AUC_{inf} (min μg/ml)	1.2 ± 0.4	0.14 ± 0.01
Dose delivered (μg/kg)	21.3 ± 7.0	–

levels were reduced to 60% by WEDD® and pharmacodynamic parameters calculated are given in Table 3. There was no difference between the calcium lowering effect of WEDD® and SC injection ($p > 0.05$) (Fig. 5). However, IV administration resulted in a higher reduction in serum calcium levels ($p < 0.05$) and higher serum SCT levels compared with other routes.

The simple zero-order input rate and clearance effectively defined the delivery pattern of SCT from the iontophoretic patch. Good correlation was observed between the experimental data and data predicted by the model. The fit of the data to the model is shown in Fig. 6 and pharmacokinetic parameters estimated are given in Table 4. Inclusion of a lag time in the model did

Table 3
Pharmacodynamic response of SCT by different routes of administration in hairless rats

Route of administration	AAEC[a]	Max % reduction in calcaemia
IV	7280.00 ± 507.31	45.53 ± 3.23
SC	4749.45 ± 713.90	38.48 ± 6.75
WEDD®	5749.02 ± 129.60	36.84 ± 4.17

[a] Area above effect curve (calculated by trapezoidal rule).

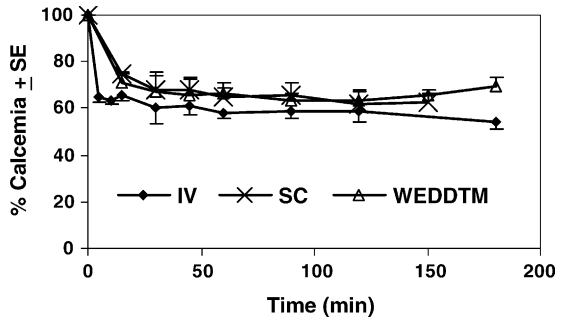

Fig. 5. Calcium lowering effect of SCT by different routes of administration in hairless rats.

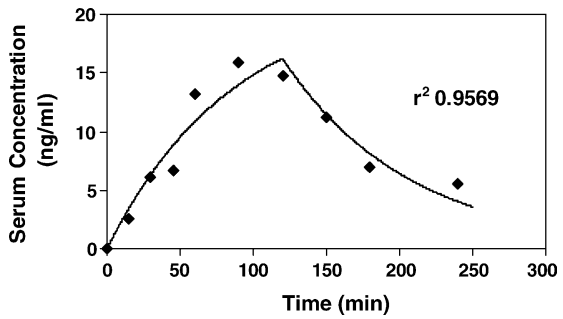

Fig. 6. Model fitting of the serum concentration vs. time profile. Closed circles represent experimental data and solid line is the prediction from the model.

not improve the AIC criteria compared with the current model. Clearance estimated by the model is similar to the clearance calculated from intravenous administration, which supports the assumptions in the calculation of dose delivered by non-compartmental analysis. Singh et al. showed for various drugs that the zero-order infusion model defines and serves practical purposes of modeling and less than perfect fit may be due to the contribution of electroosmosis during iontophoresis (Singh and Maibach, 1994; Singh et al., 1995).

Table 4
Pharmacokinetic parameters of iontophoretic delivery estimated by model regression

Parameter	Value
C_{max} (ng/ml)	8.8 ± 2.5
Cl (ml/(min kg))	16.4 ± 1.3
K (min^{-1})	0.01
Half-life (min)	54.4 ± 7.6
AUC_{inf} (min μg/ml)	1.4 ± 0.4

Clearance of SCT in hairless rats (16.8 ± 0.9 ml/(min kg)) after IV administration was higher than the clearance reported in male Sprague-Dawley rats (11.1 ± 9.0) (Song et al., 2002). Chang et al. investigated the stability of salmon calcitonin under electric current used in iontophoresis. The study concluded that the loss of calcitonin under anode or cathode is not due to degradation under the electrodes or in the electric field but may be due to adsorption within the salt bridge used in the iontophoretic setup (Chang et al., 2003). We used SCT formulation at pH 4.0 because the net positive charge would be approximately +3.8 and anodal iontophoretic flux would be aided by the electroosmosis (Santi et al., 1997). The current density (0.4 mA/cm^2) that we used is lower than the current density reported in the literature and no salt bridges were used. We evaluated the biological activity of transdermal calcitonin demonstrating that calcitonin delivered by iontophoresis is not only immunoactive but biologically active. Biological activity of SCT was evaluated by decreased serum calcium concentrations. It has been reported in the literature that 15–40% calcium lowering effect was achieved and returned to the initial value after several hours and continuous calcium lowering is typical of SCT pharamcodynamics (Thysman et al., 1994; Santi et al., 1997; Nakamura et al., 2001). Thysman et al. reported that short iontophoresis (30 min) with low current density (0.17 mA/cm^2) did not produce variation in serum calcium levels in rats compared with controls. However, they observed that short iontophoresis using 0.5 mA/cm^2 induced significant decrease in serum calcium levels and pulsed current did not have an effect on permeability of SCT through skin compared to direct current (Thysman et al., 1994). In our study, at current density 0.4 mA/cm^2 iontophoresis for 2 h, approximately 40% calcaemia reduction was achieved and is comparable with the findings in the literature. Short duration of patch applications may not produce irritation and it would substitute the invasive routes such as injections and become very patient compliant especially in case of chronic nature of the treatments such as osteoporosis. The WEDD® iontophoretic patch used is compact and can be used in clinical studies, and is on the US market for treating near surface inflammations in the Physical Rehabilitation setting. With conventional iontophoretic systems, e.g. table-top units connected by wire to patients, practical limitations exist as to patient compliance associated with being tethered to a table.

We have confirmed in this study the viability of calcitonin delivery by iontophoresis, using a device configuration that is economically amenable to a single-use, disposable patch design.

4. Conclusion

The iontophoretic patch delivered therapeutically relevant concentrations of SCT in hairless rats and delivery comparable to conventional routes such as subcutaneous injection or intravenous injection was achieved. Electronic transdermal delivery can thus be a potential platform for systemic delivery of therapeutic peptides.

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