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Transdermal iontophoretic delivery of salmon calcitonin

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Abstract

Electrically enhanced transdermal delivery of salmon calcitonin could be useful for chronic treatment of post-menopausal osteoporosis and other clinical indications as a superior alternative to parenteral delivery. Calcitonin (50 μg/ml) formulation was prepared in citrate buffer (pH 4.0). Epidermis separated from human cadaver skin was used. Most iontophoresis studies were done at a current density of 0.5 mA/cm^2. Silver/silver-chloride electrodes were used and calcitonin was found to be best delivered under the anode. The relationship between calcitonin flux and current density during iontophoresis was linear. Passive flux was zero. Flux increased with increasing concentration up to 250 μg/ml but then it levels off. Thus, transdermal delivery of salmon calcitonin may be accomplished to achieve therapeutic levels.

Keywords: Calcitonin; Iontophoresis; Transdermal; Skin transport

1. Introduction

Calcitonin is a polypeptide hormone with 32-amino acids and has a molecular weight of about 3600. It is commercially available as calcitonin human and calcitonin salmon and is used in the management of Paget's disease, hypercalcemia and post-menopausal osteoporosis (Deftos, 1998). Human calcitonin (hCT) and salmon calcitonin (sCT) both contain 32 amino acids but differ structurally at several amino acid positions. The pharmacological activity of both salmon and human calcitonin is similar, but sCT is substantially more potent on a weight basis. Since only very small quantities of a peptide can be delivered by the transdermal route, sCT was selected for these studies as its clinically effective dose is about 50 times less than that of hCT. Furthermore, hCT (but not sCT) has a marked tendency to aggregate

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in aqueous solutions (Arvinte and Drake, 1993; Bauer et al. 1995).

Because of its polypeptide nature, calcitonin is destroyed in the GI tract and must be administered parenterally, typically by subcutaneous or intramuscular injection. Non invasive methods of administration would be preferred and a nasal product has been commercialized. However, nasal delivery cannot be used if the patient has common cold or other nasal conditions. Also, the mean bioavailability via nasal delivery is low, being of the order of about 3% (Physicians' Desk Reference, Medical Economics, 1999). More importantly, treatment with calcitonin is usually prolonged and thus repeated administrations would be required. In contrast, transdermal delivery could allow for continuous input of the drug (an electric patch can be replaced every 24 h), similar to an intravenous infusion but without the need for close medical supervision or related costs. Calcitonin by itself is too large and too hydrophilic to permeate through skin so that an active enhancement mechanism, such as iontophoresis (Banga, 1998), is required. Ideally, the drug would be delivered in proportion to the applied current. Thus, continuous input could be used for chronic conditions such as paget's disease and osteoporosis, while bolus dose could be given for control of hypercalcemic emergencies. There are a few published reports on transdermal iontophoretic delivery of calcitonin (Morimoto et al., 1992; Thysman et al., 1994; Santi et al., 1997). These studies have typically used regular rats, which have a very high hair follicle density compared to human skin, an important difference since iontophoretic transport is presumed to take place via an appendageal pathway (Monteiro-Riviere et al., 1994). Also, animal skin has more proteolytic activity than human skin (Cullander et al., 1992) and is typically more permeable than human skin (Howes et al., 1996). This study provides a comprehensive in vitro investigation of the iontophoretic delivery of calcitonin across human skin. The delivery of calcitonin and other calcium regulating hormones by electroporation has been described in another paper (Chang et al., in press).

2. Materials and methods

2.1. Materials

Salmon calcitonin was purchased from Bachem Bioscience (King of Prussia, PA). ^{125}I-calcitonin (salmon) was obtained from Amersham Life Sciences (Arlington Heights, IL). Benzalkonium chloride, sigmacote, aprotinin and human serum albumin was purchased from Sigma (St Louis, MO). All other chemicals were purchased from Fisher Scientific (Pittsburgh, PA). Human cadaver skin was obtained from a skin bank and kept frozen at -80°C until ready for use.

2.2. Formulation

The donor and receptor solutions were first prepared. sCT has an isoelectric point (pI) of 10.4, so that it would exist as a cation at a lower pH. A pH of 4.0 was chosen to make the donor solution as it is sufficiently apart from the pI to get a distinct positive charge on the polypeptide so that anodal delivery can be used, as electroosmotic flow would assist delivery in this case. A pH lower than 4.0 was not chosen to avoid skin irritation. Also, lower pH can neutralize the negative charge of the skin which will reduce (or even reverse) the direction of electroosmotic flow, which will then oppose anodal delivery. Citrate buffer, 50 mM, pH 4.0 was thus used. Sodium chloride, 75 mM was added to provide Cl^- ions for the electrochemistry for the silver- silver chloride electrodes. Human serum albumin, 1 mg/ml was added to minimize adsorption of calcitonin to surfaces. Benzalkonium chloride, 0.1 mg/ml was added as a preservative. Aprotinin was added as a protease inhibitor (1000 KIU/ml) and has been shown by us to be effective in protecting calcitonin from degradation in human skin (unpublished data). A donor concentration of 50 μg/ml of calcitonin was used in all studies, unless otherwise specified. The solution was spiked with 0.25–0.50 μCi/ml of ^{125}I-calcitonin. The receptor solution was 50 mM phosphate buffer (pH 7.4) with 75 mM sodium chloride to simulate the physiological fluid.

2.3. Experimental setup and assay

Epidermis rather than full thickness skin was used in all studies, unless otherwise specified. Human epidermal membrane was separated from full thickness human cadaver skin by the widely used heat treatment method and mounted on the vertically oriented Franz transdermal diffusion cells. For iontophoresis, the current was supplied through a constant current power supply. A current density of 0.5 mA/cm^2 was used, unless otherwise specified. Silver wire was used as the anode and silver-silver chloride as the cathode. Calcitonin was delivered under the anode (unless otherwise specified) using a silver wire coiled into a 0.5 cm diameter disk and placed 1 cm above the skin surface. The cathode was extended all the way from the side arm of the Franz cell so that it comes under the skin and was at a distance of 1 cm from the skin. Samples were taken for 8 h and analyzed by liquid scintillation counting (LSC). At the end of the study, the skin was removed and washed with distilled water. It was then blotted dry, cut into fine pieces and solubilized with a tissue solubilizer. The amount remaining in the skin was then measured by LSC. Analysis of calcitonin by radioimmunoassay was also carried out (Catherwood and Deftos, 1984; Rong et al., 1997).

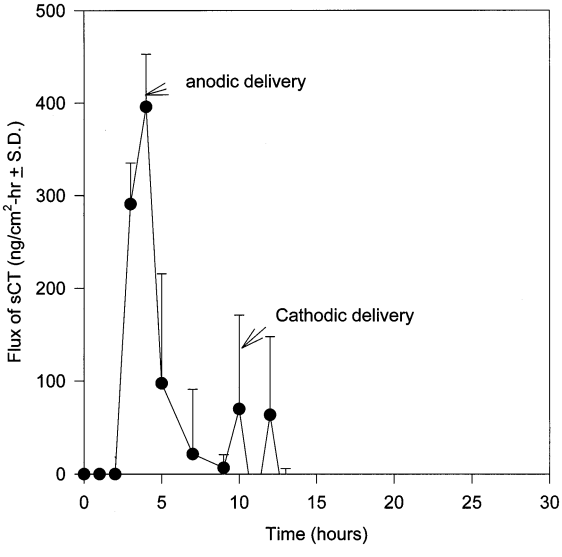

Fig. 1. Iontophoretic delivery of sCT under anodic and under cathodic polarity.

3. Results and discussion

3.1. Electrode polarity

There was no passive permeation (in absence of electric enhancement) of calcitonin across human epidermal membrane but it could be delivered by iontophoresis. Since we used a pH 4.0 buffer, an initial experiment investigated the iontophoretic delivery of calcitonin under both anode and cathode to ensure that optimal delivery is achieved under anode. This was required as the skin can undergo charge neutralization or reversal at low pH, which would reduce the magnitude of or even change the direction of electroosmotic flow. This in turn will reduce delivery under anode. The same phenomenon can also result due to the binding of a cationic drug to the skin resulting in neutralization of charge and has been reported in the literature (Delgadocharro et al., 1995). However, it was not observed in this study. It was seen that there was no permeation across human epidermis for the first 2 h when no current was applied. Permeation started when current was applied to calcitonin solution via anode from 2 to 4 h. The skin was then allowed to recover and current was reapplied at the tenth to twelfth h, but now under cathode. The results (Fig. 1) confirm that better delivery is obtained under anode. This would be expected as the molecule is strongly charged at pH 4.0, with four positive charges.

3.2. Effect of current density

The effect of increasing current density in the range 0.1–0.75 mA/cm^2 on the delivery of calcitonin (50 μg/ml) was investigated. The current was applied for 4 h. The cumulative amounts permeated increased as the current density was increased (data not shown). A current density of 0.1 mA/cm^2 was inadequate to deliver any detectable levels of calcitonin but the delivery increased with increasing current density beyond

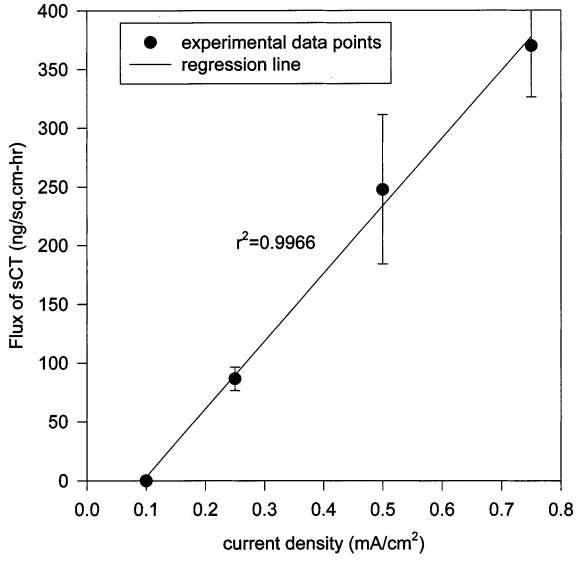

Fig. 2. sCT flux as a function of increasing current density.

this point. The lack of detectable delivery at 0.1 mA/cm^2 may be due to inadequate driving force to deliver calcitonin through the skin. The flux at h 4 was then plotted against the current density and a linear relationship with a very high correlation coefficient (r^2, 0.99) was observed (Fig. 2). This suggests that the delivery of calcitonin can be programmed by adjusting the current density (within limits). Since the current is easily controlled by electronics, the technology provides a convenient means to control the rate of delivery of drugs. However, the current density and current intensity cannot be indefinitely increased as it will irritate and/or damage the skin and also produce unpleasant electrical sensation. In general, 0.5 mA/cm^2 is often stated to be the maximum current density that should be used on humans. All subsequent studies have thus used this current density. At the end of the study, the skin was dissolved as described in Section 2. The amount remaining in the skin was then measured as a function of current density (Fig. 3). As can be seen, a current density of 0.1 mA/cm^2 did drive the drug into the skin, even though it did not deliver it across the skin as discussed. The amount remaining in the skin increased with current density, but not much beyond 0.5 mA/cm^2. This could be because the skin is getting saturated, though other mechanisms may also be possible and are not clear.

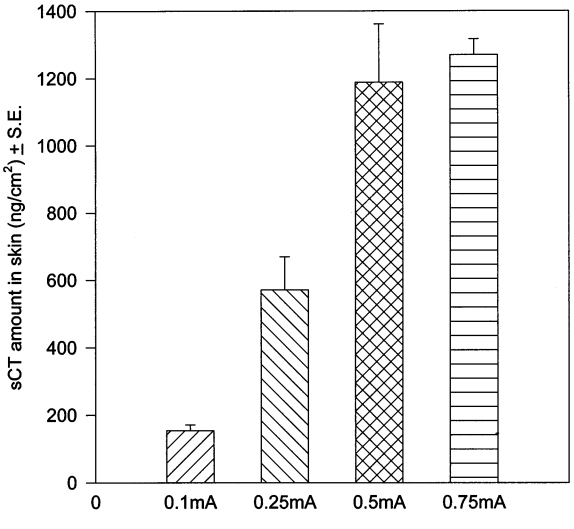

Fig. 3. The amount of sCT remaining in the skin at the end of the study as a function of increasing current density.

3.3. Effect of competitive ions on iontophoretic delivery

Competitive ions should be minimized as they will reduce the efficiency of delivery. However, some ions are necessary such as the ones from citrate buffer here which is required to maintain the pH. Some sodium chloride is also necessary to drive the electrochemistry of silver/silver chloride electrodes:

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

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

The reaction at anode in the absence of chloride ion may be written as:

$$Ag \rightarrow Ag^+ + e^-$$

In accordance with the above equation, we observed that in the absence of sodium chloride, the donor solution (containing anode) became cloudy as the silver anode gradually dissolved in solution. The time to cloudiness in the donor solution was 30 and 110 min for 5 mM and 20 mM of sodium chloride, respectively. In order to allow at least 4 h of iontophoresis, a 75 mM concentration of

sodium chloride was required and was used in all studies. Next, the citrate buffer concentration was investigated. The iontophoretic delivery of calcitonin at 5, 10 and 25 mM citrate buffer strength was investigated. The corresponding cumulative amounts permeated at 8 h were 2430 ± 280, 1750 ± 540 and 2650 ± 770 ng/cm^2 $\pm$ S.E., respectively. The differences in permeation were not statistically significant between groups (t-test, $P > 0.2$). Thus, it seems that the calcitonin flux was not significantly affected by the change in citrate concentration. This most likely resulted due to the presence of sodium chloride which predominantly contributed to ionic strength so that the changes resulting from citrate buffer strength were overshadowed. A pH drift was observed in the donor solution with low ionic strength buffers. Only 50 mM strength provided enough buffer capacity to maintain the pH in the donor for the duration of the experiment. Thus, 50 mM buffer was used in all investigations.

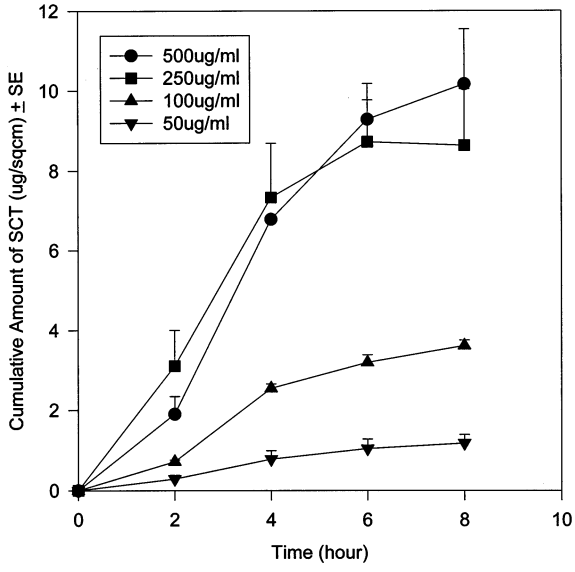

Fig. 4. Cumulative amounts permeated as a function of donor concentration of sCT.

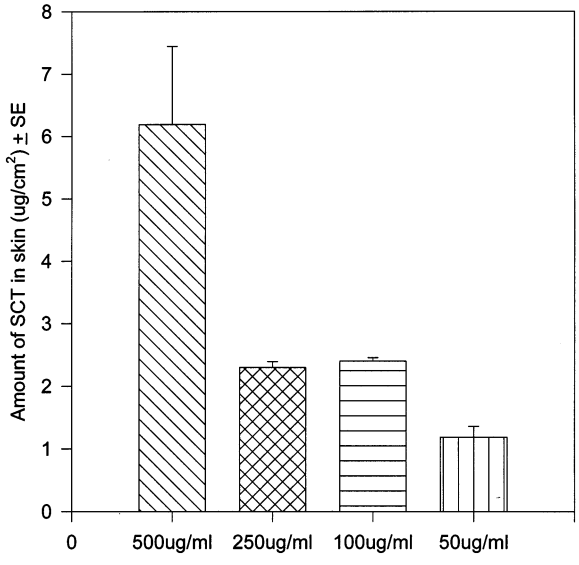

Fig. 5. The amount of sCT remaining in the skin at the end of the study as a function of increasing donor concentration.

3.4. Effect of drug concentration on iontophoretic permeation

An increase in the drug concentration in the formulation will typically result in higher iontophoretic delivery. Increasing concentration will increase iontophoretic delivery up to some point but at still higher concentrations, the flux may become independent of concentration. For conventional molecules, this could be because the boundary layer gets saturated with the drug while the bulk donor solution is still not saturated. The same is not expected to be the case for low levels of peptides. However, our results (Fig. 4) did not show a linear correlation between permeation and donor concentration (50–500 μg/ml). The data between 100 and 500 μg/ml is statistically significant (t-test; $P = 0.002$) but not between 250 and 500 μg/ml. A plot of the flux shows that there is a linear increase up to 250 μg/ml but then it levels off. The delivery efficiency was estimated by the amount permeated divided by the donor concentration and was found to be 2% for 50, 3.6% for 100, 3.6% for 250 and 2% for 500 μg/ml. Thus the optimum concentration should be between 100 and 250 μg/ml. The lack of a linear relationship over the concentration range selected could be due to a greater ionic interaction between positively charged sCT and negatively charged skin at higher concentrations, though the exact mechanism is not clear. The amount remaining in the skin at the end of the study as a function of donor

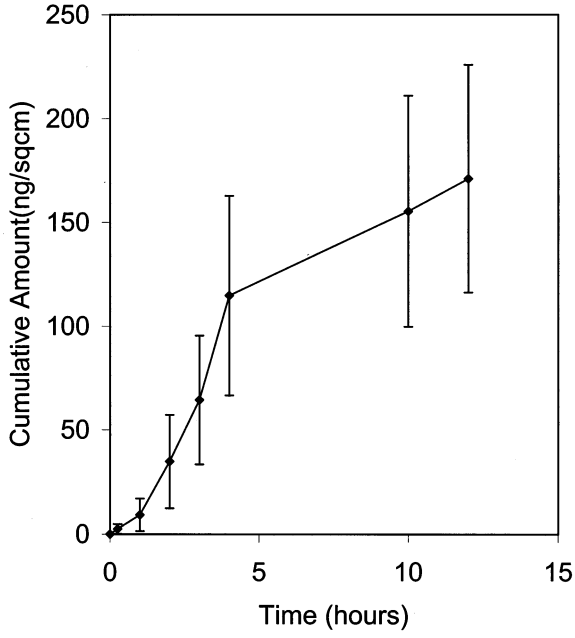

Fig. 6. Cumulative amount of sCT permeated when imidazole buffer (pH 6.0) was used for the donor solution.

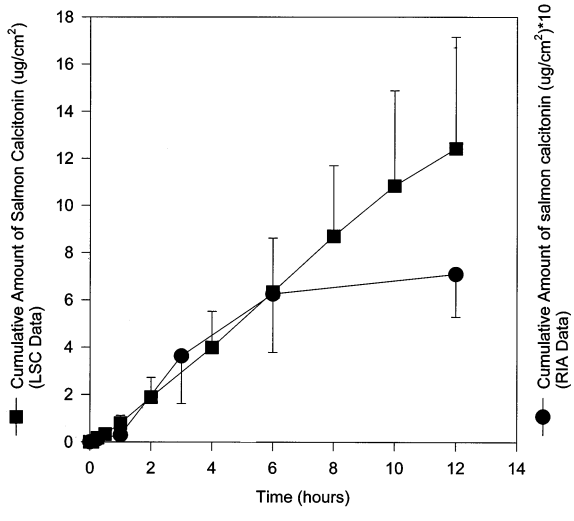

Fig. 7. permeation of sCT across skin as measured by LSC or RIA.

concentration was also measured (Fig. 5) and was about 1–2 μg/cm^2 except at 500 μg/ml which resulted in 6 μg/cm^2. This suggests that more calcitonin is retained in the skin as the concentration is increased beyond a certain limit. The permeation flux of sCT at 50 and 250 μg/ml concentration was compared (graph not shown). It was observed that a steady state flux was not observed at 50 μg/ml but an almost immediate input followed by steady state flux (within 15 min) results when the higher 250 μg/ml concentration was used.

3.5. Transport efficiency and stability considerations

In our donor solution formulation, we use sodium chloride to provide the Cl^- ions necessary to drive the electrochemistry of silver/silver chloride electrodes. The accompanying Na^+ ions are expected to give a competitive effect, thus reducing the efficiency of delivery. In an attempt to improve delivery efficiency, we used imidazole buffer where pH is adjusted with HCl, thus providing Cl− ions without the addition of extraneous Na^+ ions. However, this buffer is unable to provide pH 4.0 so that a pH of 6.0 was used instead. The concentration of salmon calcitonin was 50 μg/ml, same as most earlier experiments. The cumulative amount permeated with 4 h of iontophoresis (Fig. 6) is about 10-fold less than similar experiments with citrate buffer/sodium chloride. Thus, we actually observed a decreased efficiency rather than an increase in efficiency. This is most probably related to the reduced charge density of salmon calcitonin at pH 6.0 in comparison to pH 4.0. In separate studies, we have addressed various stability issues related to the storage stability of calcitonin, its stability under electric field and its stability upon passing through the skin. Calcitonin was found to be stable under the electric field used (unpublished data). The amounts permeated through skin as analyzed by LSC were compared to analysis by radioimmunoassay (RIA). A similar profile was seen with both analysis, establishing that immunoactive calcitonin is being delivered across the skin (Fig. 7). The magnitude of permeation, however, was less in immunoassay. This is not entirely unexpected as LSC will typically overestimate delivery as there is usually some degradation taking place during delivery. The in vivo studies in rats further established the bioactivity of the

calcitonin delivered through skin (unpublished data).

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