Effect of Current Magnitude and Drug Concentration on Iontophoretic Delivery of Octreotide Acetate (Sandostatin®) in the Rabbit

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The effect of current magnitude and drug concentration on transdermal iontophoretic delivery of octreotide acetate (Sandostatin®) was examined in the rabbit. Plasma samples were collected over 24 hours and octreotide concentrations were determined by a radioimmunoassay. Without an electrical current, negligible plasma concentrations of octreotide were obtained. Following initiation of iontophoresis, plasma concentrations of octreotide increased rapidly, although did not sustain at a plateau level during the dosing period. Octreotide concentrations declined rapidly after removal of the device. Increasing the electrical current from 50 $\mu A/cm^2$ to 150 $\mu A/cm^2$ yielded a proportional increase in the delivery. Increasing the drug concentration in the device from 2.5 mg/mL to 5 mg/mL resulted in approximately proportional increase in plasma octreotide concentrations; however, further increase in plasma concentrations was not observed for drug concentrations beyond 5 mg/mL. Iontophoretic delivery at the conditions which yielded the highest octreotide concentrations in this study (5 mg/mL solution at 150 $\mu A/cm^2$ for 8 hours) yielded an apparent bioavailability (which represents an underestimate of the absolute bioavailability determined when the patches are run to exhaustion) of approximately 8%.

KEY WORDS: iontophoresis; octreotide; transdermal; peptide; rabbit.

INTRODUCTION

Octreotide acetate (Sandostatin®, Sandoz), a synthetic octapeptide (1), is a somatostatin (growth hormone) analog which is used clinically for the treatment of acromegaly (2), pancreatic endocrine tumors (3), and the carcinoid syndrome (4). Octreotide is currently available as a parenteral dosage form for subcutaneous injection (5), while other more convenient routes of drug delivery are being investigated. Oral dosing of octreotide was found to yield relatively low bioavailability (6). Passive transdermal delivery is a possible alternative. However, octreotide, similar to many other peptides, is a charged molecule. Therefore, the flux of the peptide across the skin, especially through the lipophilic layer of stratum corneum, is limited.

Transdermal drug delivery via iontophoresis, the process by which ionic or charged molecules are transported in the presence of an electric current, was first proposed in the 18th century (7). Theoretically, iontophoresis can provide a viable means of delivering peptides and proteins because of the ionic nature of these compounds. It has been demonstrated that protein drugs, such as insulin (8) and thyrotropin-releasing hormone (9), can be successfully delivered into the systemic circulation via iontophoresis. The objective of this study was to examine the iontophoretic delivery of octreotide, using the rabbit as the animal model. The effects of current magnitude and drug concentration on the systemic delivery of the drug were investigated.

MATERIALS AND METHODS

Drug Substance

Octreotide (MW = 1019.3), (D)-Phe-Cys-Phe-(D)Trp-Lys-Thr-Cys-Thr(ol), was prepared as the acetate salt. At pH 5.5, i.e. the approximate pH of the formulation, the peptide exhibits a net 2+ charge. Different concentrations of octreotide (2.5, 5, 10, and 15 mg/ml of free base equivalents) were prepared in a solution containing 100 mM NaCl. The iontophoretic patches were affixed to animals using peripheral adhesives and were overwrapped using a porous medical tape. The tape was then covered with a mesh jacket which held the controller. The skin contact area of the patches was 1 cm^2. A continuous DC current was applied using an external constant current source. The patch system, similar to those reported previously (10,11), comprised of a separate anode and cathode. The anode was of a two compartment design, with the electrochemical reaction chamber and a lower skin contacting drug reservoir. Cyclic voltammetry indicated that octreotide was electrochemically stable under the anticipated system parameters. Ag/AgCl electrochemistry was utilized in both the anode and cathode compartments to avoid pH shifts. Immediately prior to use the drug reservoir, which consisted of a sponge-like polymeric matrix, was filled with a solution containing octreotide at the designated concentration in 100 mM NaCl. A 100 mM NaCl solution was also added to the cathode reservoir immediately before application.

Animal Experimentation

All animal studies were approved by the Sandoz Animal Care and Use Committee. Seven New Zealand White female rabbits, each weighing 3–4 kg, were used in two different studies. The patches were applied dorsally to the back of the animals. The site of application was clipped on the day before dosing. In the first study, iontophoretic patches containing approximately 110 μL of octreotide solution were applied over a 6-hour period to 3 rabbits at three different current levels (50, 100, and 150 $\mu A/cm^2$) on three different study days, using a crossover design. The concentration of the octreotide solution was 5 mg/mL. Subsequently, on a fourth study day, each animal was administered a passive

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control patch containing the same concentration of drug, but in the absence of an electrical current. Serial blood samples were obtained via the marginal ear vein at 0, 0.5, 2, 4, 6, 6.5, 7, 8, and 24 hours after initiation of iontophoresis. On a fifth study day, an intravenous bolus dose containing 50 μg octreotide (base equivalent) was administered via the marginal ear vein. Blood samples were collected at 0, 0.083, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, and 4 hours following intravenous dosing. All study days were separated by a 6-day washout period.

In the second study, iontophoretic patches containing approximately 110 μL of octreotide solution at varying concentrations (2.5, 5, 10, and 15 mg/mL) of octreotide dose were applied to four rabbits over an 8-hour period on four different study days, using a crossover design. A current of 150 μA/cm^2 was used. Serial blood samples were obtained via the marginal ear vein at 0, 0.5, 2, 4, 6, 8, 9, 10, and 24 hours after initiation of iontophoresis. As in the first study, there was a 6-day washout period between treatments.

Determination of Plasma Octreotide Concentrations

The plasma fraction was obtained from blood samples collected in both studies, and the concentration of octreotide was determined using a radioimmunoassay specific to the intact peptide, based on the method described by Kutz et al. (5). Polyclonal antiserum was obtained from a rabbit that had been immunized with a conjugate of octreotide and bovine serum albumin, emulsified with Freund's complete adjuvant. Radiolabeled ligand was prepared by iodination of the D-Tyr analog of octreotide and subsequently purified by HPLC. Antiserum, tracer, and plasma (unknown, standard, or control) samples were incubated in phosphate buffered saline (pH 7.2) for 18–24 hours at 4°C. Antiserum-bound drug was separated from free drug by the addition of a chilled plasma-coated charcoal slurry. After centrifugation, the ^{125}I-radioactivity of the supernatant was counted using a Packard, Cobra II, gamma counter. The limit of quantification in these studies was 39 pg/mL.

Pharmacokinetic Analysis

The peak concentration (C_{max}) and time of peak concentration (t_{max}) following each iontophoretic administration were recorded. The area under the plasma concentration-time curve (AUC) during 0–24 hours was calculated using the linear trapezoidal rule without extrapolation, since octreotide concentration approached the detection limit within 24 hours in nearly all studies. The average plasma concentration data after intravenous dosing was fitted with a monoexponential equation. The AUC following intravenous administration was calculated using the log-trapezoidal method and was extrapolated to infinite time (12). The clearance (CL) and the volume of distribution (V) of octreotide were obtained by the following equations:

$$CL = \frac{D_{iv}}{AUC_{iv}}$$

$$V = \frac{D_{iv}}{C(0)}$$

where D_{iv} is the intravenous dose, AUC_{iv} is the area-under-the concentration-curve, and C(0) is the extrapolated concentration at zero time following intravenous dosing.

In addition, the rate and extent of iontophoretic delivery were estimated by a finite difference numerical deconvolution method (13,14) implemented using IMSL/IDL (15). The mean plasma concentrations from the intravenous group in the first study was fitted with a monoexponential equation to yield an impulse response function. The mean plasma concentrations resulting from the iontophoretic administration were then deconvoluted using the impulse response function to provide an estimate of the cumulative amount of drug that was available in the systemic circulation.

RESULTS

Figure 1 shows the mean plasma concentrations of octreotide following iontophoresis at different current magnitudes. Delivery of octreotide was enhanced by the presence of electrical currents, when compared to the passive control group. Plasma concentrations increased rapidly following initiation of iontophoresis, and decreased rapidly at the end of dosing. As the current increased from 50 to 150 μA/cm^2, the average plasma concentrations of octreotide increased. The pharmacokinetic parameters of octreotide are summarized in Table I. Despite substantial inter-animal variability, average C_{max} and AUC values appeared to increase with increasing current magnitudes. However, no difference in t_{max} was apparent between groups.

Figure 2 shows the plasma concentrations of octreotide with increasing drug concentrations in the patch. As in the first study, plasma concentrations of octreotide increased rapidly following initiation of iontophoresis, and rapidly declined after removal of the patches. There appeared to be no changes in t_{max} and C_{max} with different drug concentrations in the patches (Table I). The AUC increased almost proportionally from 2.5 mg/mL to 5 mg/mL, but showed no further increase at octreotide concentrations of 10 mg/mL and 15 mg/mL. This is further illustrated by the ratios of AUC in individual animals following iontophoretic administration of the 5 mg/mL, 10 mg/mL, and 15 mg/mL solutions, as compared to the 2.5 mg/mL solution. A two-fold increase in octreotide concentration (5 mg/mL) resulted in doubling (2.04

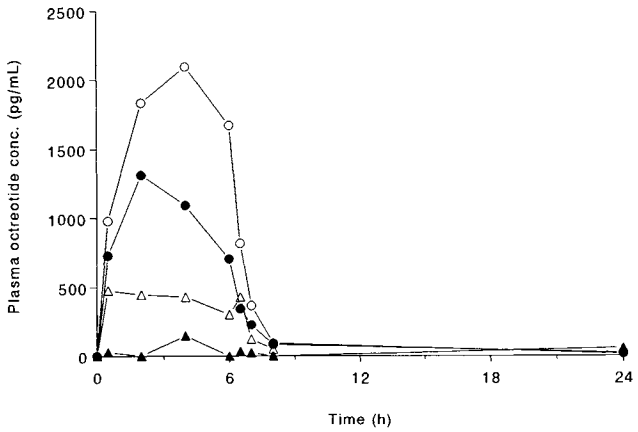

Fig. 1. Plasma concentrations of octreotide following 6-hour iontophoretic delivery at 0(-▲-), 50(-△-), 100(-●-), and 150(-○-) μA/cm^2, using a 5 mg/ml octreotide dose solution (mean of 3 rabbits).

Table I. Pharmacokinetic Parameters of Octreotide in Two Different Iontophoresis Studies, Compared to an Intravenous Dose, in the Rabbit

Study	N	Current ($\mu A/cm^2$)	Drug concentration (mg/mL)	Dose (μg)	C_{max} (pg/mL)	t_{max} (h)	AUC (pg · h/mL)	AUC/DOSE[1] (pg · h/mL)/μg	Apparent bioavailability[1] (%)
Intravenous	3	—	—	50	—	—	21300 ± 2850	426	—
Current	3	50	5	550	763 ± 553	3.0 ± 3.1	3250 ± 2930	5.91	1.4
magnitude	3	100	5	550	1340 ± 709	2.7 ± 1.2	7350 ± 5740	13.4	3.2
	3	150	5	550	2140 ± 877	3.7 ± 1.5	12100 ± 4670	22.0	5.2
Dose	4	150	2.5	275	2260 ± 375	3.1 ± 2.4	9930 ± 2790	36.1	8.5
concentration	4	150	5	550	2340 ± 504	2.5 ± 1.0	18900 ± 2090	34.3	8.1
	4	150	10	1100	1950 ± 870	4.5 ± 3.0	13700 ± 6200	12.4	2.9
	4	150	15	1650	1700 ± 1500	5.5 ± 2.5	10300 ± 7870	6.22	1.5

[1] Mean values are used for calculation.

± 0.69) of the AUC, whereas 4- and 6-fold greater octreotide concentrations (10 mg/mL and 15 mg/mL) only yielded AUC ratios of 1.47 ± 0.69 and 1.03 ± 0.41, respectively.

Following intravenous dosing, octreotide was rapidly eliminated from the systemic circulation. The elimination half life of octreotide was approximately 23 min. The AUC of octreotide was 21300 pg · h/mL (Table I), and the clearance and volume of distribution of the drug were estimated to be 2.35 L/h and 1.43 L, respectively. The variability in AUC was relatively small compared to that observed following transdermal iontophoresis administration.

The cumulative amount of octreotide delivered to the systemic circulation was determined by deconvolution, using the monoexponential fit of the intravenous data as the impulse response model. The results for the current-dependence and the dose concentration-dependence studies are shown in Figures 3a and 3b, respectively. The amount of octreotide delivered to the systemic circulation was approximately proportional to the magnitude of the current (Figure 3a). Delivery also appeared to be proportional to the concentration of octreotide in the dosing solution, up to 5 mg/mL (Figure 3b). However, increasing the drug concentration above 5 mg/mL resulted in a gradual decrease in the amount of octreotide reaching the systemic circulation. Indeed, similar AUC values of octreotide were obtained for the 2.5 mg/mL and the 15 mg/mL solutions.

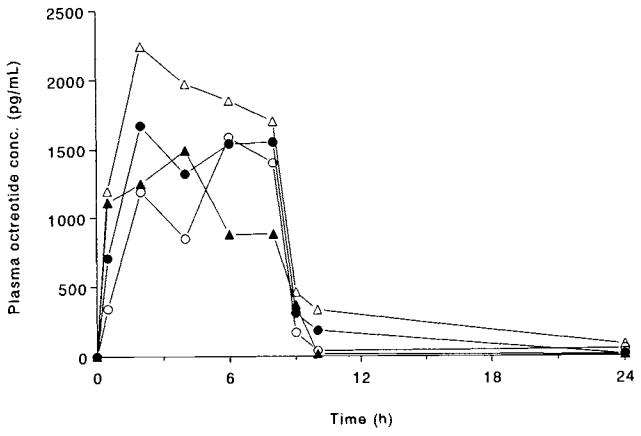

Fig. 2. Plasma concentrations of octreotide following 8-hour iontophoretic delivery at 150 $\mu A/cm^2$, using octreotide dose solutions of 2.5(-▲-), 5(-△-), 10(-●-), and 15(-○-) mg/ml. (mean of 4 rabbits).

DISCUSSION

Since octreotide is indicated for chronic diseases such as acromegaly, pancreatic tumors, and gastrointestinal cancers, it is desirable to explore more convenient alternative methods of drug delivery, other than the existing parenteral form. Oral doses of octreotide yielded limited bioavailability in humans (6), probably due to low permeation rate and pos-

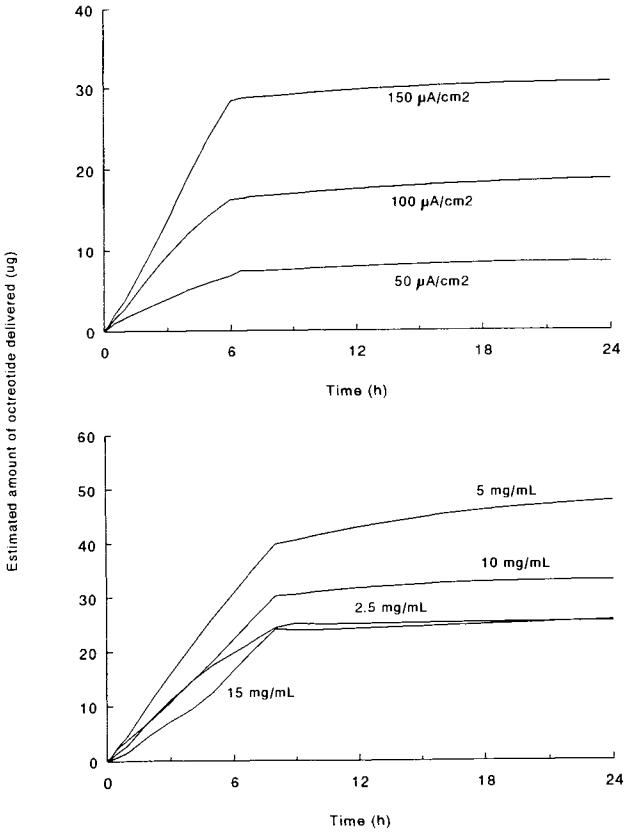

Fig. 3. Estimated amount of octreotide delivered into the systemic circulation at (a) different current magnitudes and (b) different drug concentrations in the dose solutions, using deconvolution.

sible degradation of the drug in the gastrointestinal tract. In fact, it was found that a specific transport system in the jejunum is responsible for the enteral absorption of octreotide, and the presence of bile decreases the extent of absorption (16). Therefore, oral dosing of the drug is probably not the preferred route of delivery. In contrast, transdermal iontophoresis provides a convenient means of systemic delivery of charged compounds. In the presence of an electrical current, the permeation rate of octreotide across the skin could be enhanced, due to the ionic nature of the peptide (17). Moreover, drug concentrations can be maintained over a longer duration using this mode of sustained delivery. This is especially desirable for octreotide which, despite being more stable than its endogenous analog (18), exhibits a relatively short half-life of 1.5–2 h in both the rat (19) and in man (5,20).

The findings from this study clearly demonstrate that compared to passive transdermal delivery, iontophoresis substantially increased the amount of octreotide delivered into the systemic circulation. Moreover, no skin irritation was observed during the studies. These results suggest that iontophoresis is a feasible method to deliver octreotide. The amount of octreotide delivered increased as a function of the applied current. This is consistent with Faraday's Law, which states that ion flux is proportional to the magnitude of the current. On the other hand, increasing the concentration of octreotide in the dosing solution above 5 mg/mL did not increase the systemic availability of the drug. In fact, further increases in octreotide concentrations appeared to yield lower circulating levels of drug. This finding cannot be readily explained at present. Continuing work to investigate the physicochemical behavior of high concentrations of octreotide in the iontophoretic device is currently in progress. It is interesting that at the optimal conditions for iontophoresis used in this study, i.e. higher current and lower dose concentrations, smaller inter-animal variability in AUC were obtained (Table I).

The apparent bioavailability of the iontophoretic patches was calculated from the ratio of the dose-normalized AUC following iontophoresis and intravenous administration, as shown in Table I. The apparent bioavailability increased from 1.4% to 5.2% when the current was increased from 50 $\mu A/cm^2$ to 150 $\mu A/cm^2$. Upon increasing octreotide concentrations from 2.5 mg/mL to 15 mg/mL, the apparent bioavailability decreased from 8.5% to 1.5%. At the optimal conditions that yielded the highest circulating drug levels (5 mg/mL octreotide solution at 150 $\mu A/cm^2$ for 8 hours), the apparent bioavailability was 8.1%. It should be noted that the apparent bioavailability determined here represents an underestimate of the "absolute bioavailability", which is determined when the patches are run to exhaustion.

After the patches were removed, the rate of decline of plasma octreotide concentrations appeared to be similar to that obtained following intravenous administration, suggesting that the delivery of octreotide into the circulation was promptly terminated. At 24 hours after initiation of patch application (16 or 18 hours after termination of iontophoresis), octreotide concentrations declined to undetectable levels. These findings suggest no accumulation of the drug in the skin or subsequent release of drug into the systemic circulation. It appears that in the absence of an electrical current, passive diffusion of octreotide through the skin is negligible, as observed for the control group in this study.

If the rate of iontophoretic delivery is the rate-limiting step of absorption and is zero-order in nature, one would expect to observe a plateau of plasma octreotide concentrations near the end of the dosing period, assuming that there is no time-dependent change in the dispositional characteristics of the drug. However, it is evident that in both studies, plasma octreotide concentrations declined before the patches were removed. This observation suggests that in these studies, iontophoretic delivery may not be truly zero-order throughout the dosing period, and may be time-dependent. Since the patches were designed to contain excess drug, it is unlikely that the observation was due to the depletion of the patches. However, it is possible that the associated increase in hydration of the rabbit skin, due to occlusion and iontophoresis-driven electro-osmosis of water into the skin, may alter the apparent transference number of octreotide compared to other ions which were present in the delivery system (e.g., Na^+, Cl^-, or OAc^-). A decrease in the apparent transference number is reflective of a decrease in the fraction of the applied current responsible for delivering the drug, therefore resulting in a time-dependent decrease in the delivery rate. Upon attainment of "skin equilibrium", a steady-state concentration profile should be obtained as the hydration stabilizes. While this observation cannot be readily explained, it should be noted that decreased iontophoretic peptide fluxes with increasing concentration have been observed in vitro for leuprolide (21) and nafarelin (22).

In summary, iontophoretic delivery provides a viable means of increasing the transdermal permeability of octreotide into the systemic circulation. The systemic availability increased as a function of the magnitude of the current and also upon increasing drug concentrations, up to 5 mg/ml.

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