Transdermal Iontophoretic Delivery of Triptorelin *In Vitro*

YANNIC B. SCHUETZ,[1,2] AARTI NAIK,[1,2] RICHARD H. GUY,[1] EVELYNE VUARIDEL,[3] YOGESHVAR N. KALIA[1,2]

[1]School of Pharmaceutical Sciences, University of Geneva, Geneva CH-1211, Switzerland

[2]Centre Interuniversitaire de Recherche et d'Enseignement, ''Pharmapeptides,'' Site d'Archamps, F-74160 Archamps, France

[3]Debiopharm Galenic Unit, CH-1196 Gland, Switzerland

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ABSTRACT: The feasibility of delivering triptorelin ([*D*-Trp^6]LHRH) by transdermal iontophoresis was evaluated *in vitro*. Peptide electrotransport at different current densities and donor concentrations was measured across porcine ear skin. The concomitant delivery of an electroosmotic marker enabled calculation of the respective contributions of electromigration (EM) and electroosmosis (EO) to iontophoretic delivery. At a given concentration (3 mM), a threefold increase in current density produced a corresponding increase in the cumulative amount of peptide present in the receptor compartment. Conversely, doubling the concentration to 6 mM produced a twofold reduction in the amount of peptide delivered, partly due to a concentration-dependent inhibition of EO. EM was revealed to be the predominant transport mechanism, accounting for 80% of overall delivery. Finally, despite the inhibition of EO, the results indicate that application of an iontophoretic current of 0.8 mA over a relatively small contact area (4 cm^2) would provide a delivery rate of 36 μg/h, largely sufficient for therapeutic requirements. J Pharm Sci 94:2175–2182, 2005

Keywords: transdermal iontophoresis; triptorelin; electroosmosis; electroosmosis inhibition

INTRODUCTION

Transdermal delivery has been proposed as a route for administering peptide and protein therapeutics on the grounds that it offers a viable alternative to the conventional, and inconvenient, administration by parenteral injection. However, given that peptides are often charged and of high molecular weight, their passive transdermal delivery is not feasible. Hence, different strategies have been developed to overcome the skin's excellent barrier properties in a transient and reversible fashion.[1,2] Iontophoresis offers the advantage of providing a controlled and noninvasive delivery method that has been extensively investigated.[3] The two main transport mechanisms during iontophoresis are electromigration (EM; direct effect of the applied electric field on the charged species) and electroosmosis (EO; convective solvent flow in the anode-to-cathode direction, as a consequence of the skin's net negative charge at physiological pH).

A distinguishing feature of iontophoresis is that, in contrast to other enhancement technologies, it acts primarily on the molecule itself. That is, enhanced delivery is not due to increased passive drug transport subsequent to barrier disruption: the driving force is supplied by the applied electric field. Furthermore, iontophoresis enables customized therapy: the drug-input rate

Evelyne Vuaridel's present address is Department of Pharmacy & Pharmacology, University of Bath, Claverton Down, Bath BA2 7AY, United Kingdom.

Correspondence to: Yogeshvar N. Kalia (Telephone: +33 450 31 50 24; Fax: +33 450 95 28 32; E-mail: yogi.kalia@pharm.unige.ch)

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can be adapted to the individual needs of each patient, or to the phase of treatment in response to disease progression, by modulating the intensity of the applied current. In addition, different permutations of the current profile enable continuous or pulsatile delivery and other more complex drug input profiles, for example, drug input at a basal rate followed by an "on-demand" bolus as in patient-controlled analgesia.

Control over the input profile is particularly valuable for drugs that have different pharmacological effects depending on their input rate, such as luteinising hormone-releasing hormone (LHRH) and human parathyroid hormone (PTH). For example, pulsatile administration of LHRH, every 60–90 min, is used in the treatment of female infertility due to hypothalamic hypogonadism,[4] in order to stimulate gonadotrophin release. In contrast, prolonged continuous application of LHRH and its analogues, suppresses gonadotrophin secretion and is the underlying mechanism in the therapy of hormone-dependent cancers.[5]

Several studies investigating the iontophoretic transdermal transport of LHRH, and its more potent and longer-acting analogues have been conducted.[6–11] Some of the most relevant results were obtained with [*D*-Leu^6,Pro^9-NHET] LHRH (leuprolide, Lupron^{TM}), which was successfully delivered *in vivo* in humans:[8,12] a peak LH response similar to that obtained with subcutaneous injection was measured after iontophoretic delivery of leuprolide.[12] Interestingly, these results were accompanied by some unexpected behavior, not consistent with theory: namely, that increasing the dose did not result in enhanced delivery.[8,13] Similar findings have been described for other LHRH analogues and have been attributed to the association of the lipophilic cations with the membrane, neutralizing the intrinsic negative charge of the skin and leading to a significant reduction in the electroosmotic transport of the peptide.[10,14]

The aim of this study was to evaluate the feasibility of delivering triptorelin ([*D*-Trp^6]LHRH, Decapeptyl®) by transdermal iontophoresis and to investigate the transport mechanisms involved. Co-iontophoresis of acetaminophen was used to deconvolve the contributions of EO and EM and to report on the impact of triptorelin iontophoresis on skin permselectivity. The effect of current density and peptide donor concentration on delivery was assessed. Triptorelin transport was also compared to that of a tripeptide (Ac-*D*-Trp-Leu-Arg-NH_2) corresponding to its sequence at positions.[6–8]

MATERIALS AND METHODS

Chemicals

Triptorelin (MW = 1311.5 Daltons) pGlu-His-Trp-Ser-Tyr-*D*-Trp-Leu-Arg-Pro-Gly-NH_2, in the form of the acetate salt was a generous gift (Debiopharm Galenic Unit, Gland, Switzerland). Ac-Tyr-(*D*-Trp)-Lys-NH_2 and Ac-(*D*-Trp)-Leu-Arg-NH_2 were custom-synthesized (NeoMPS SA, Strasbourg, France). De-ionized water (resistivity > 18 MΩ/cm^2) was used to prepare all solutions.

Analytical Procedures

Triptorelin was quantified by high-performance liquid chromatography. The HPLC system comprised a pump (Waters 600E System Controller, Waters Corporation, Milford, MA), dual wavelength UV detector (Waters 2487 Dual λ Absorbance Detector), autoinjector (Waters 717plus Autosampler), and was equipped with a C18 PartiSphere column (4.6 mm i.d., 12.5 cm long, 5 μm particle size) (Whatman, Inc., Florham Park, NJ) maintained at 40°C. The mobile phase (25% acetonitrile and 75% triethylaminephosphate buffer solution pH 2.3) delivered at a flow rate of 1 mL/min was degassed in-line (Waters In-Line Degasser AF). Triptorelin was detected at 280 nm. The RSD of the repeatability was less than 1% and the quantification limit was 130 ng/mL. The tripeptides were analyzed using the same conditions but with a mobile phase consisting of acetonitrile: triethylaminephosphate buffer, pH 2.3 (12:88). The RSD of the repeatability was less than 1% and the quantification limit was 55 ng/mL.

Acetaminophen was assayed by high-performance liquid chromatography using a Hypersil BDS C8 column (150 mm × 4.6 mm, Supelco®, Sigma-Aldrich Chimie Sarl, France) maintained at 40°C. The mobile phase (92% water and 8% acetonitrile adjusted to pH 3.5 with acetic acid) was delivered at a flow rate of 1 mL/min. Acetaminophen was detected by its UV-absorbance at 243 nm. The RSD of the repeatability was less than 1% and the quantification limit was 22 ng/mL.

Skin Preparation

Porcine ears were obtained from the local abattoir shortly after sacrifice. After cleaning under cold running water, the whole skin was removed carefully from the outer region of the ear and separated from the underlying cartilage with a

scalpel. Given that epidermis is a well-established model for transdermal drug delivery, and that dermatomed skin might act as an artifactual reservoir and binding site,[15–17] all experiments with triptorelin were performed using heat-separated epidermis.[18] Pieces of fresh full-thickness skin were immersed in water at 58°C for 2 min after which the epidermis was carefully separated from the dermis, wrapped in Parafilm™ and maintained at −20°C for no longer than a period of 2 months before use.

Iontophoresis

The skin was mounted in three-compartment vertical diffusion cells (area: 0.73 cm^2), the design of which has been described in detail elsewhere.[19] Except in the case of the stability experiments, the anode was isolated from the donor solution via a salt bridge (100 mM Tris/Trizma HCl in 3% agarose) to minimize competition between the peptide and the electrolytes necessary for the anodal reaction. Anodal, cathodal, and receptor compartments contained a solution of 25 mM Tris/Trizma® HCl normal saline buffered to pH 7.4. Triptorelin (3 mM unless otherwise stated) was solubilized in 20 mM Tris/Trizma® HCl (pH 7.4). In addition to the peptide, the donor compartment (1 mL) always contained 15 mM acetaminophen.

Constant current iontophoresis was used in all the experiments. The current ranging from 0.15 to 0.5 mA/cm^2 was applied for 4 to 8 h via Ag/AgCl electrodes connected to a power supply (Kepco, Flushing, NY).

Stability Experiments

The susceptibility of triptorelin to degradation by porcine skin enzymes was assessed in the following manner. Epidermal sections were mounted in diffusion cells and the receptor compartment was filled with a 5 μM triptorelin solution, in 25 mM Tris/Trizma® HCl-buffered (pH 7.4) normal saline; both the anodal and cathodal compartments were filled with 1 mL of 25 mM Tris/Trizma® HCl-buffered (pH 7.4) normal saline. The concentration of intact triptorelin in the receptor compartment was determined after application of a 0.15 mA/cm^2 current for 8 h.

Quantification of Electroosmotic Solvent Flow

Acetaminophen is a neutral hydrophilic compound, which is primarily transported through the skin by EO. It was therefore included in the donor compartment formulation (15 mM) as a marker for the magnitude of convective flow (15 mM). For each experiment, an inhibition factor (IF) was calculated according to the following equation:

$$IF = [Q_{A\text{-}8h,control}]/[Q_{A\text{-}8h,peptide}] \quad (1)$$

where $Q_{A\text{-}8h,control}$ is the amount of acetominaphen transported into the receptor phase during 8 h of iontophoresis when no peptide was present in the donor solution and $Q_{A\text{-}8h,peptide}$ is the corresponding quantity when triptorelin was iontophoresed.

Effect of Current Density and Triptorelin Concentration

To evaluate the effect of current density on both triptorelin transport and inhibition of EO, current densities of 0.15, 0.3, and 0.5 mA/cm^2 were applied for 8 h using a donor peptide concentration of 3 mM.

The iontophoretic delivery of a higher concentration (6 mM) at 0.5 mA/cm^2 for 8 h was also investigated to determine the impact of the donor concentration on the electroosmotic flow and overall peptide transport.

Data Treatment

All measurements were performed in at least triplicate, using skin samples originating from different pig ears. Outliers, determined using the Grubbs test, were discarded.

RESULTS AND DISCUSSION

Triptorelin Stability in the Presence of Skin

Investigation of the *in vitro* stability of triptorelin in the presence of epidermis revealed a degradation of $9 \pm 2\%$ after 8 h suggesting that it was quite resistant to proteolysis. Peptide susceptibility to enzymatic degradation is obviously dependent on the amino acid sequence; for comparison, under the same conditions, again using heat-separated porcine epidermis, the somatostatin analogue, vapreotide, suffered significantly greater metabolism ($68 \pm 11\%$).[17] The difference in stabilities also demonstrates that enzymatic activity is maintained in spite of the skin preparation procedure. Amidation of the C-termini

ensures that both peptides are protected against carboxypeptidases. The cyclic *p*Glu and *D*-Phe residues at the N-termini protect triptorelin and vapreotide, respectively, against aminopeptidase activity. Thus, endopeptidases or reductases targeting the disulphide bridge, present in vapreotide, but not in triptorelin, might also be active in the skin. Extrapolation of these results to the *in vivo* situation is difficult and not only because of interspecies differences. Although enzymatic activity is expected to be higher in viable tissues (the freezing process is reported to reduce metabolic activity of skin samples,[20]) *ex vivo* skin preparation procedures might increase the release of intracellular enzymes.

Triptorelin Delivery and the Effect of Current Density

The quantities of triptorelin transported across porcine epidermis from a 3 mM donor solution after 4, 6, and 8 h of anodal iontophoresis (at a current density of 0.5 mA/cm^2) are depicted in Figure 1. The cumulative amounts permeated after 6 and 8 h enable estimation of the flux at 7 h (22 μg/cm^2/h). The iontophoretic transport rate of [*D*-Trp^6,Pro^9-NHET] LHRH, which has almost complete sequence homology with triptorelin ([*D*-Trp^6] LHRH), across hairless mouse skin *in vitro* was very similar (17 μg/cm^2/h) at the same current density (0.5 mA/cm^2) and donor concentration (3 mM).[7] Although iontophoretic delivery of triptorelin across human and rabbit skins has been reported,[11] quantitative comparison is rendered difficult by the differences in experimental conditions and the variability of the data.

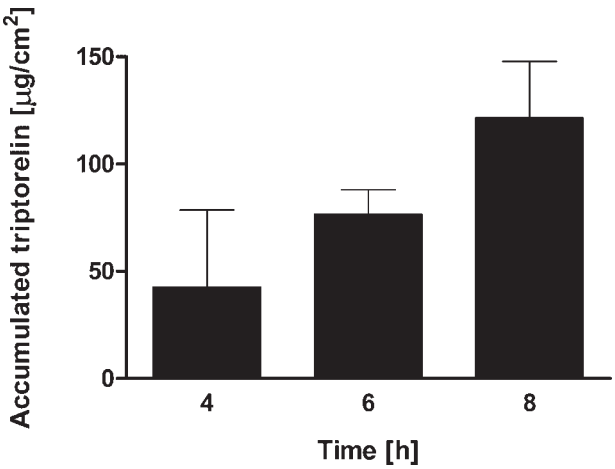

Figure 1. Cumulative transport of triptorelin across porcine skin *in vitro* following iontophoretic current application at 0.5 mA/cm^2 for 4, 6, and 8 h, respectively. The formulation in the anodal compartment comprised 3 mM triptorelin in 20 mM Tris/Trizma at pH 7.4.

The iontophoretic flux (J) of a charged species is the sum of two transport mechanisms—electromigration (J_{EM}) and electroosmosis (J_{EO}), assuming that passive diffusion is negligible:

$$J = J_{EM} + J_{EO} \quad (2)$$

$$J_{EM} = \frac{t_{\#} \cdot I}{Z \cdot F} \quad (3)$$

According to Faraday's law, J_{EM} is proportional to product of the applied current density (I) and the transport number ($t_{\#}$), where Z and F represent the charge and Faraday's constant, respectively. Theoretical models describing the dependence of the transport number on the concentration and mobility of the charge carriers in the system are rendered complicated since the skin is a (negatively) charged membrane (Kasting and Keister have applied the electroneutrality approximation to the Nernst–Planck equation.[21]) Since the electroosmotic flow, from anode-to-cathode under physiological conditions increases with applied current density, [22] both transport mechanisms, and hence the total iontophoretic flux are related to the applied current. This is illustrated by the data in Table 1, which shows that a threefold increase in current density (0.15–0.5 mA/cm^2) resulted in a corresponding increase in the cumulative amount of triptorelin in the receiver compartment (35 ± 9 to 120 ± 30 μg/cm^2). In contrast, the increase in current produced a sharp decrease in acetaminophen transport; a sixfold reduction was observed at 0.5 mA/cm^2, indicative of considerable EO inhibition.

Linear correlations between flux and current density have been reported for nonpeptidic compounds as well as for small peptides (e.g., TRH[23] and Threo-Lys-Pro.[24]) For larger peptides, although an increased current density usually results in increased permeation, straightforward linear correlations are not always observed. For example, a poor correlation was observed between DGAVP (9-desglycinamide, 8-arginine-vasopressin) flux and applied current; a more than sixfold increment in current density did not even double the flux.[25] However, it was shown that increasing the applied current density from 0.1 to 0.5 mA/cm^2 produced an almost threefold increase in the flux of the structurally-related peptide [*D*-Trp^6,Pro^9-NHET] LHRH.[7]

Table 1. Effect of Current Density on the Cumulative Amount of Triptorelin (3 mM) Permeated After an 8 h Current Application and its Impact on Skin Permselectivity

Current Density [mA/cm^2]	Triptorelin Transport [μg/cm^2]	Acetaminophen Transport [μg/cm^2]	Inhibition Factor[a]
0.15	35 ± 9	35 ± 26	1.0 ± 0.8
0.3	60 ± 30	10 ± 5	3.6 ± 1.8
0.5	120 ± 30	8 ± 3	6 ± 3

[a]Inhibition factors (IF) calculated according to Equation 1.

Contributions of Electromigration and Electroosmosis to Triptorelin Transport

Since acetaminophen is a neutral hydrophilic molecule with negligible passive skin permeability, its iontophoretic transport is almost exclusively due to electrically-induced convective solvent flow and, as such, its transport can be used to report on EO. During iontophoresis, the velocity (V_w) of the current-induced water flow (units of cm/h, equivalent to a permeability coefficient) across the skin can be estimated using Eq. 4 [26]:

$$V_w = J_{ace}/C_{ace} \quad (4)$$

where J_{ace} and C_{ace} are the flux and donor concentration of acetaminophen, respectively. It follows that a measurement of J_{ace} at known C_{ace} allows V_w to be determined. It is then possible to calculate the EO contribution to the flux of the peptide by multiplying V_w by its concentration in the donor solution ($C_{peptide}$) [27]:

$$J_{EO} = V_w \cdot C_{peptide} \quad (5)$$

Two assumptions are implicit in this analysis: (a) that drug and acetaminophen are transported in a similar fashion by convective solvent flow, and (b) that electroosmotic transport of the marker molecule is proportional to its concentration in the solvent.

Co-iontophoresis of acetaminophen (15 mM) with triptorelin (3 mM) at 0.5 mA/cm^2, for 8 h, resulted in an acetaminophen flux of 18 nmol/cm^2/h; this was used to calculate V_w using Eq. 4. The quantity of triptorelin transported by EO could then be estimated by substitution of V_w (1.2×10^{-3} cm/h) into Eq. 5. Given the measured total flux of triptorelin under the same conditions (17 nmol/cm^2/h), Eq. 2 allows assignment of the relative contributions of EO and EM to the total iontophoretic flux. The analysis reveals that EM is the dominant transport mechanism for triptorelin, accounting for ~80% of overall transport ($J_{EO} = 3.6$ nmol/cm^2/h; $J_{EM} = 13.4$ nmol/cm^2/h). It is worth noting that in the absence of any inhibition, the maximum theoretical EO contribution ($J_{EO,max}$, that is, assuming $V_w \sim 4.8 \times 10^{-3}$ cm/h) to the iontophoretic delivery of triptorelin (3 mM at 0.5 mA/cm^2) would only be ~14 nmol/cm^2/h; implying that for this decapeptide (MW ~1311 Daltons), EM would still account for ~50% of iontophoretic transport.

Acetaminophen transport in the presence of triptorelin clearly demonstrated that the latter inhibited EO (IF of 6 ± 3), albeit to a much lesser extent than certain other peptides. For example, vapreotide iontophoresis under equivalent conditions resulted in an IF of 50 ± 30.[17] Vapreotide is doubly charged (due to the lysine side chain and the free N-terminal) and this probably favors the interaction with negatively charged sites in the skin and accounts for its greater propensity to inhibit EO. Furthermore, the presence of a disulphide bridge in vapreotide probably contributes to a more compact three-dimensional structure than triptorelin; the preferred conformations may orient the key moieties so as to favor interaction with the skin's binding sites, and hence facilitate EO inhibition.

Are Peptide Fragments Useful Predictors of Transport?

Nafarelin ([*D*-Nal(2)^6] LHRH) and the tripeptide (*D*-Nal(2))-Leu-Arg (representing the amino acids at positions 6–8) were demonstrated to be equipotent EO inhibitors.[14] In contrast, while triptorelin caused EO inhibition (IF = 6 ± 3), co-iontophoresis of its "peptide motif" Ac-(*D*-Trp)-Leu-Arg-NH_2 with acetaminophen failed to result in a corresponding effect (IF = 1.1 ± 0.2) (Fig. 2). Iontophoresis of the tripeptide Ac-Tyr-(*D*-Trp)-Lys-NH_2, derived from the residues at positions 3–5 in vapreotide, under the same experimental conditions, also produced almost no inhibition of EO (IF = 1.5 ± 0.5) compared to the parent peptide

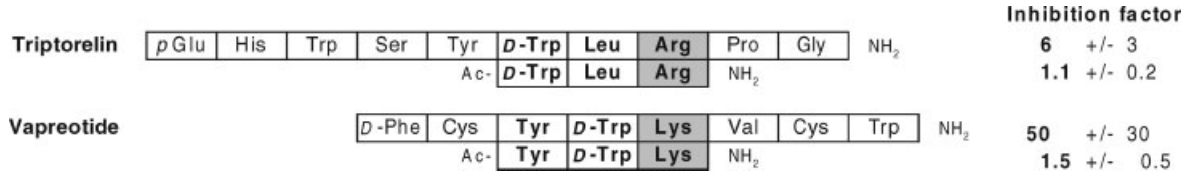

Figure 2. Inhibition factors (IF) of triptorelin, vapreotide, and their constituent tripeptides after iontophoretic current application at 0.5 mA/cm^2 for 8 h. The formulation in the donor compartment comprised 3 mM peptide in 20 mM Tris/Trizma at pH 7.4.

(IF = 50 ± 30).[17] Hence, the occurrence and extent of this phenomenon cannot, as a rule, be accurately predicted from the behavior of the structural motif presumed responsible for the skin interaction. It should be noted that interspecies differences (the triptorelin and vapreotide studies were conducted with porcine skin whereas nafarelin and (*D*-Nal(2))-Leu-Arg data were obtained using hairless mouse skin) may also play a role in the interpretation of mechanistic data. This is further illustrated by the observation that the dependence of iontophoretic propranolol delivery on donor concentration (in the presence of competing ions), across these two membranes, is different.[3,27]

Effect of Increasing Triptorelin Concentration on Peptide Transport and Electroosmosis

The impact of drug concentration on iontophoretic flux is a commonly studied experimental parameter. According to Eq. 3, an increase in the formulation's drug-load should result in an increase in the EM component and hence in the total drug flux (with the assumption that the formulation concentration is equivalent to that present in the supposed aqueous transport pathways within the membrane). Indeed, when the donor formulation contains background electrolyte, a source of competing ions, the initial linear dependence that is observed between flux and drug concentration dwindles as concentration increases: once the product of the drug concentration and mobility (see Eq. 3) is in sufficient excess of the corresponding values for the competing ions, the flux becomes independent of drug concentration. Triptorelin contains the (*D*-Trp-Leu-Arg) sequence at positions 6–8 in its primary structure; this sequence corresponds to the oligopeptide motif (hydrophobe-hydrophobe-cation) hypothesized to be responsible for the "anomalous" iontophoretic behavior observed with nafarelin, leuprolide, and octreotide upon increasing the donor concentration. In a second series of transport experiments with twice the triptorelin concentration (6 mM) in the donor compartment, only 60 ± 40 μg/cm^2 of triptorelin was measured in the receptor compartment, compared to 120 ± 30 μg/cm^2 with the lower donor concentration (3 mM); a twofold increase of peptide in the formulation produced a twofold decrease in delivery. The simultaneous administration of acetaminophen as an electroosmotic marker, allowed the IF to be calculated. The IF values at 3 and 6 mM triptorelin concentrations were 6 ± 3 and 10 ± 5, respectively, substantiating the inhibitory effect of this peptide on the convective solvent flow. However, given that EO accounted for only ~20% of iontophoretic transport at a donor concentration of 3 mM, the increased EO inhibition cannot alone explain the twofold reduction in the cumulative amount delivered: the increase in donor concentration must also impact on EM. Hence, it seems likely that other interactions, perhaps involving the formation of triptorelin aggregates, which would hinder peptide delivery, must occur within the transport pathways.

The separate effects of aggregation and inhibition could be represented by modification of Eq. 2:

$$J_T^{EXP} = (1-\alpha)J_{EM}^{PRED} + (1-\beta)J_{EO}^{NOINHIB} \qquad (6)$$

where α and β represent the degree of aggregation and EO inhibition, respectively. Increasing aggregation would reduce drug mobility and predominantly affect EM; β, which is proportional to IF, would negatively impact upon convective solvent flow. Thus, peptide accumulation could occur without affecting EO. At the same time, peptides could strongly inhibit EO and still have that as the major transport mechanism, if they also exhibit a high degree of aggregation.

Can Therapeutic Amounts of Triptorelin Be Delivered by Transdermal Iontophoresis?

The "bottom line" of any feasibility study with a therapeutic molecule is to determine whether sufficient drug can be delivered to achieve the

desired pharmacological effect. For triptorelin, the plasma concentration required for biochemical castration is ~1.7 nmol/L (equivalent to ~2.2 ng/mL).[28] Given that the total body clearance in healthy individuals is ~200 mL/min,[29] the target input rate that must be achieved to maintain the steady state triptorelin concentration necessary for durable biochemical castration is ~26 μg/h. The measured triptorelin flux of 22 μg/cm^2/h (at 7 h) signifies that at the current density used in these experiments (0.5 mA/cm^2), it would be entirely feasible to deliver therapeutic levels of triptorelin with a patch application area of less than 2 cm^2. As reported above, triptorelin delivery increases essentially linearly with current; hence, the measured peptide flux can be normalized by the applied current density to obtain a measure of mass transport per unit current per unit time; the "current-normalized" flux for triptorelin is 45 μg/mA/h. A current density of 0.5 mA/cm^2 is at the upper limit of the range generally considered acceptable for human use, and is probably unsuitable for prolonged application. The current-normalized flux can be used to calculate the application area necessary to deliver therapeutic amounts of triptorelin at lower, and better tolerated, iontophoretic current densities. For example, the application of a total iontophoretic current of 0.6–0.8 mA, over a 4 cm^2 contact area, equivalent to more acceptable current densities of 0.15–0.2 mA/cm^2, would be sufficient to provide therapeutic delivery rates (27–36 μg/h).

CONCLUSIONS

These preliminary *in vitro* studies illustrate the contrasting effects of current density and triptorelin concentration on delivery. At a given concentration (3 mM), a threefold increase in current density produced a corresponding increase in the cumulative amount of peptide transported across porcine epidermis. Conversely, doubling the concentration to 6 mM produced a twofold reduction in the amount of peptide delivered following iontophoresis for 8 h at 0.5 mA/cm^2. Although theory would suggest equivalent effects upon increasing current or concentration, the experimental results reveal that this is not always the case. Thus, formulation parameters must be carefully selected to optimize the delivery of complex molecules. Quantification of acetaminophen transport in the presence of triptorelin revealed that EM was the predominant transport mechanism, accounting for ~80% of overall delivery. The acetaminophen data also revealed that triptorelin was capable of a concentration-dependent EO inhibition. Nevertheless, the degree of inhibition was insufficient to explain the inverse dependence of transport on peptide concentration, suggesting the involvement of peptide aggregation. Despite the EO inhibition and putative aggregation phenomena, the cumulative amount of triptorelin delivered and the estimated iontophoretic flux suggest that drug input rates sufficient to achieve plasma levels capable of ensuring prolonged biochemical castration may be attainable using transdermal iontophoresis.

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