# Physical and Chemical Enhancement of Transdermal Delivery of Triptorelin

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## INTRODUCTION

Triptorelin is a decapeptide analogue of the luteinizing hormone-releasing hormone, which currently is administered as implantable microparticles for the treatment of sex hormone-dependent tumors and other benign gynecologic disorders (1).

Transdermal delivery could be an attractive method for triptorelin administration. Several studies have been performed on the transdermal transport of luteinizing hormone-releasing hormone and its analogues. In particular, Miller *et al.* (2) studied the penetration of triptorelin through hairless mouse skin in the presence of pulsatile iontophoresis. The results showed a steady-state rate capable of providing therapeutic doses, with continuous current application.

Because the practical use of transdermal iontophoresis would benefit from short current application times, we focused mainly on the use of "shots" of current followed by passive diffusion.

In the present work, we studied the *in vitro* passive and iontophoretic transdermal penetration of triptorelin to establish optimal conditions for this route of administration. To increase the amount of drug permeated, chemical enhancers and iontophoresis were used.

## MATERIALS AND METHODS

### Materials

Triptorelin acetate [molecular weight (net) = 1311.5] was a gift from Lipotec (Barcelona, E) ($pKa_1 = 7.2$; $pKa_2 = 9.5$; $pKa_3 = 12$) (3). Lauric acid, egg L-α-phosphatidylcholine, sodium azide, and agarose were purchased from Sigma Chemical Co. (St. Louis, MO); Transcutol® (diethyleneglycolmonoethylether) was obtained from Gattefossé (Milan, Italy). All other products were of analytical grade.

### High-Performance Liquid Chromatography (HPLC)

For HPLC analysis, an isocratic pump (C-R6A, Shimadzu, Japan) and a UV-Vis spectrophotometric detector (SPD-10A, Shimadzu, Japan) were used. Chromatographic conditions were as follows: column Spherisorb C18 250 × 4.6 mm (Waters, Milford, MA), acetonitrile, and water (40/60 v/v) with 0.1% (v/v) trifluoracetic acid as mobile phase, flow rate 1 $mL \cdot min^{-1}$, and UV detection at 220 nm. The peak area was linear with concentration in the interval 0.01–0.1 $\mu g \cdot mL^{-1}$ with a correlation coefficient of 0.996. The reproducibility was 2.8%, the limit of quantification 0.008 $\mu g \cdot mL^{-1}$, the tailing factor 1.03 ± 0.12, and the number of theoretical plates was 530.

### Stability

The stability of triptorelin solutions (3 $\mu g \cdot mL^{-1}$) in the media used for permeation experiments, in the presence of electric current and lauric acid, was tested at room temperature. The solutions obtained were analyzed by HPLC at time zero, after 24 h, and after 48 h.

### Permeation Experiments

Permeation experiments were performed on rabbit ear skin and abdominal human skin using vertical diffusion cells (area: 0.6 $cm^2$). The receptor chamber was kept at 37°C and filled with NaCl 0.9% (w/v) containing 0.02% (w/v) sodium azide. The experiments lasted for 48 h in the conditions illustrated in Table I. The current was applied by means of a constant current generator (Iono1, Cosmic, Pesaro, Italy), using salt bridges and Ag/AgCl electrodes. The current (intensity: 0.3m A) was applied for 1 or 2 h, and then donor reservoir was left in contact with the skin up to 48 h. Because triptorelin is positively charged (3), anodal iontophoresis was applied.

## RESULTS AND DISCUSSION

### Stability

Table II illustrates the stability of triptorelin in the different vehicles used for permeation experiments. The concentrations at various times were expressed as percentages of the initial concentration. The stability of the peptide in buffer (pH 5) with mannitol 3% (w/v) was very good, in agreement with the literature data (3). The peptide also showed an acceptable stability in the receptor medium. Furthermore, triptorelin was not degraded after 2 h of current application (intensity 0.3 mA) in buffer (pH 5).

In contrast, the stability of triptorelin in the donor solution containing lauric acid (pH 3.5) was poor despite the good stability data reported in literature for this pH value. (3). Because triptorelin stability was good in the ethanol/water mixture, the degradation was probably due to the presence of lauric acid. Further studies are necessary to clarify the degradation mechanism of triptorelin in the presence of lauric acid.

### Permeation across Rabbit Ear Skin

Rabbit ear skin was chosen for a preliminary study because it was characterized and proven to be a reasonable model for transdermal iontophoresis (4). The permeation of triptorelin from NaCl 0.9% (w/v) solution and from Transcu-

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**Table I.** Experimental Conditions and Triptorelin Permeation Data

| Skin | Vehicle (300 μL) containing triptorelin 10 mg $mL^{-1}$ | Iontophoresis | Amount permeated after 48 h (μg $cm^{-2}$) |
|---|---|---|---|
| Rabbit ear skin | NaCl 0.9% (w/v) | — | nd[a] |
| Rabbit ear skin | NaCl 0.9% (w/v) with Transcutol 10% (w/v) | — | nd |
| Rabbit ear skin | NaCl 0.9% (w/v) with egg L-α phosphatidylcholine (10% w/v) | — | nd |
| Rabbit ear skin | ethanol/water (60/40) with 4% (w/v) lauric acid | — | 6.9 ± 1.7 |
| Rabbit ear skin | pH 5 acetate buffer 0.1 M with 3% (w/v) mannitol | Anodal, 1 h 0.3 mA | 23.6 ± 13.0 |
| Rabbit ear skin | pH 5 acetate buffer 0.1 M with 3% (w/v) mannitol | Anodal, 2 h 0.3 mA | 16.1 ± 6.6 |
| Rabbit ear skin | ethanol/NaCl 0.9% (w/v) (60/40) with 4% (w/v) lauric acid | Anodal, 1 h 0.3 mA | 8.2 ± 2.4 |
| Abdominal human skin | NaCl 0.9% (w/v) | — | nd |
| Abdominal human skin | ethanol/water (60/40) with 4% (w/v) lauric acid | Anodal, 1 h 0.3 mA | 137.4 ± 32.2 |
| Abdominal human skin | pH 5 acetate buffer 0.1 M with 3% (w/v) mannitol | Anodal, 1 h 0.3 mA | 183.1 ± 100.3 |

[a] nd = below limit of quantification.

tol® and egg L-α-phosphatidylcholine donors did not show any measurable transport of the drug. The only enhancer that was able to generate a measurable flux of the peptide through rabbit skin (Fig.1) was lauric acid, which is known to modify the structure of the stratum corneum barrier (5). Furthermore, laurate ion is reported in literature as being able to form ion pairs with positively charged molecules, thus increasing their solubility in the stratum corneum (5). In fact, lauric acid solution has a pH of 3.5, and triptorelin is present in its cationic form at this value of pH.

We then investigated the ability of anodal iontophoresis to enhance triptorelin transport, focusing on the use of a shot of current to increase drug transport. From the profiles obtained (see Fig. 1), it can be observed that the application of electric current for 1 hour increased the amount of triptorelin transported across the skin (see Table I). The application of electric current for 2 h did not further increase triptorelin transport.

A possible mechanism to explain the post-iontophoretic triptorelin flux is represented by the formation of a drug reservoir within the skin during iontophoresis, although it is unlikely that triptorelin can accumulate in the skin because of its very hydrophilic nature. It is more reasonable to suppose that iontophoresis causes an increase in skin permeability, which persists after the current is switched off. This mechanism is supported by the data of Volpato *et al.* (6) and of Turner *et al.* (7); however, the biologic modifications involved in skin permeability enhancement are not defined (8).

The physical and chemical penetration strategies were then combined to evaluate the possibility of a synergism as reported by Bhatia *et al.* (9). An electric current was applied to a donor solution of triptorelin with lauric acid 4%. Figure 1 shows that no significant skin penetration improvement was obtained. The amount permeated was not significantly different from passive diffusion from the solution containing lauric acid, and it was significantly lower than that transported by iontophoresis alone. This discrepancy with the literature data may be due to the use of rabbit skin because the effect of chemical enhancers on the skin's permeability to peptides is highly dependent on the skin model used (10).

### Permeation across Human Skin

The conditions that originated a measurable flux through rabbit ear skin were then tested on abdominal human skin. Figure 2 shows the permeation profiles obtained with lauric acid solution in comparison with NaCl 0.9% (w/v) solution. In agreement with rabbit skin data, passive diffusion from a

**Table II.** Percentage of Triptorelin Remaining after 24 and 48 Hours in Different Vehicles and in the Presence of Current

| Time (h) | pH 5 0.1M acetate buffer with 3% (w/v) mannitol | $EtOH/H_2O$ 60/40 lauric acid 4%(w/v) | $EtOH/H_2O$ 60/40 | Electric current (i = 0.3 mA; 2 h)[a] | Receptor medium[b] |
|---|---|---|---|---|---|
| 0 | 100 | 100 | 100 | 100 | 100 |
| 24 | 100 ± 2 | 89 ± 4 | 95 ± 3 | 100 ± 3 | 98 ± 2 |
| 48 | 100 ± 1 | 77 ± 6 | 94 ± 3 | 100 ± 2 | 93 ± 2 |

[a] In pH 5 0.1M acetate buffer with 3% (w/v) mannitol.
[b] NaCl 0.9% (w/v) with sodium azide 0.02% (w/v).
Initial Drug Concentration was 3 μg $mL^{-1}$. Mean ± SEM (n = 3).

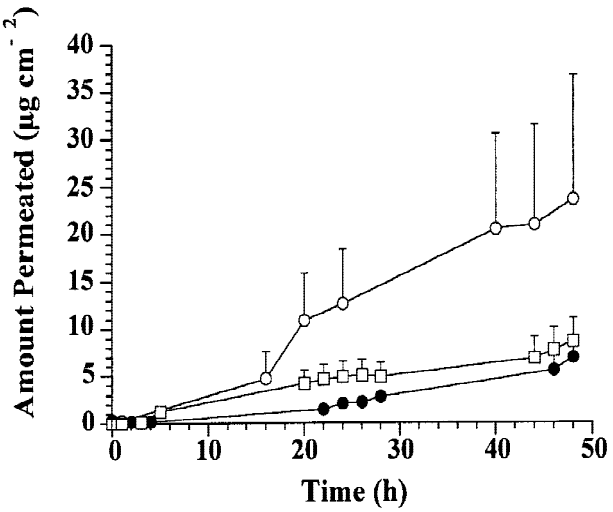


**Fig. 1.** Permeation profiles of triptorelin across rabbit ear skin, from donor solutions containing the drug at 10 mg · ml$^{-1}$ in ethanol:water (60:40) containing lauric acid 4% (closed circles); pH 5 buffer solution, and iontophoresis applied for 1 h (open circles); ethanol:water (60:40) containing lauric acid 4% and iontophoresis applied for 1 h (open squares). Mean values ± standard error of the mean; (n ≥ 4).

NaCl 0.9% (w/v) solution was below the limit of detection, whereas in the presence of lauric acid triptorelin, permeation was promoted. The permeation profile is characterized by a very long time lag (about 10 h) attributed to the use of full-thickness skin.

One hour of anodal iontophoresis on human skin, followed by passive diffusion, gave a total amount of drug permeated at 48 h, which was not significantly different from that obtained with chemical enhancement (see Table I).

Human skin proved more permeable than rabbit skin, even though in the case of iontophoresis, the difference was not statistically significant due to the high variability encountered in the data. This difference in permeability does not find any comparison in literature, where rabbit skin generally is reported as having a higher passive permeability (11,12). However, the samples of rabbit skin referred to in literature were samples of shaved dorsal skin with a hair follicle density of 229 ± 57 cm$^{-2}$ (11), whereas the hair follicle density of the skin used in this work (inner ear) is comparable to that of human skin (11 ± 1 cm$^{-2}$). Additionally, Wester and Maibach (12) demonstrated that the difference in permeability between human and rabbit skin is highly dependent on the lipophilicity of the permeant and is lower for hydrophilic molecules. Nonetheless, it is worth mentioning that Hirvonen *et al.* (13) registered a higher permeability of rabbit ear skin in comparison with human for indomethacin, 5-fluorouracil, and propranolol.

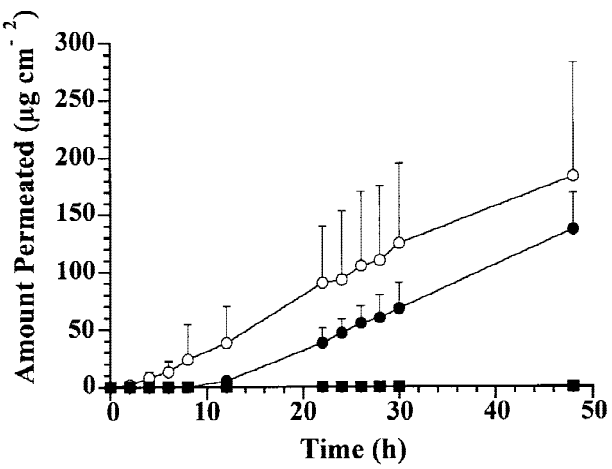


**Fig. 2.** Permeation profiles of triptorelin across full thickness human skin, from donor solutions containing the drug at 10 mg · ml$^{-1}$ in NaCl 0.9% (w/v) solution (closed squares); ethanol:water (60:40) containing lauric acid 4% (closed circles); pH 5 buffer solution and iontophoresis applied for 1 h (open circles). Mean values ± standard error of the mean (n ≥ 4).

Given that in the post-iontophoretic experiments a steady-state flux could not be reasonably calculated even after the time lag had elapsed, the flux across human skin was calculated between 12 and 48 h. The estimated rates were 3.82 ± 0.85 and 4.09 ± 1.82 μg · cm$^{-2}$h$^{-1}$ for lauric acid and iontophoresis, respectively. The values were not different, but the time lag of passive permeation was significantly reduced by iontophoretic application.

As a reference to discuss the flux data obtained in the present work, the minimum amount of triptorelin necessary to maintain therapeutic plasma levels was calculated. From the total clearance (14) and concentration at steady state (15), the minimum input rate of triptorelin was calculated as 0.76 μg · h$^{-1}$. Considering an application area of 20 cm$^2$, the required flux will be 0.0378 μg · cm$^{-2}$h$^{-1}$. Because both current and lauric acid stimulated transdermal fluxes are two orders of magnitude higher that the estimated average flux needed for therapeutical use of triptorelin, transdermal application of this compound is very well feasable, even with smaller flux area, or lower drug concentration in the patch.

## CONCLUSIONS

Transdermal administration of triptorelin using lauric acid or iontophoresis can be taken in consideration as an alternative to the parenteral route. Lauric acid was shown to have a good impact on the flux of triptorelin, but from our experiments, we showed a negative influence on the stability of the drug. Moreover, lauric acid has been claimed to cause skin irritation; therefore, it might be necessary to search for another promoter. Anodal iontophoresis, on the other hand, was shown to be an applicable method to improve triptorelin permeation, and current applications could be limited to 1 h because the enhancing effect continued for 48 h.

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