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Note

Stability of triptorelin in the presence of dermis and epidermis

Yogeshwar G. Bachhav, Yogeshvar N. Kalia *

School of Pharmaceutical Sciences, University of Geneva and University of Lausanne, 30 Quai Ernest Ansermet, 1211 Geneva, Switzerland

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ABSTRACT

An important issue with respect to the transdermal delivery of peptides is their stability during transit through the epidermis and dermis before entry into the systemic circulation. The objective of the present study was to evaluate the effect of epidermal and dermal tissue on the stability of the luteinizing hormone releasing hormone superagonist, triptorelin. The decapeptide was dissolved in PBS (pH 7.4) and placed in contact with (i) heat separated epidermis (HSE), (ii) dermatomed skin (0.75 mm; DS) and (iii) full thickness skin (FTS) and the extent of peptide biotransformation monitored as a function of time by HPLC. The results showed that triptorelin was metabolized when in contact with each of the skin tissues. However, there were marked differences with respect to the extent of peptide degradation. Triptorelin was least stable in the presence of FTS. After 3 h exposure to HSE, DS and FTS, the extent of triptorelin degradation was 15.0 ± 6.0%, 64.8 ± 9.9% and 100%, respectively. After 24 h, further triptorelin degradation had occurred in the samples in contact with HSE and DS—with 51.3 ± 6.0% and 87.8 ± 4.4%, respectively, of the peptide being degraded. The chromatograms revealed the presence of a degradation peak at a higher retention time than the parent molecule—most probably the free acid.

Transdermal delivery has received considerable attention as a non-invasive means to administer therapeutic peptides (Schuetz et al., 2005a). However, there are few reports on peptide stability in the presence of skin; in particular, with respect to the contribution of the individual skin layers to peptide degradation. In addition to using a variety of skin models, studies have also employed different tissues (e.g., heat separated epidermis or dermatomed skin). In this work, the objective was to evaluate the effect of different porcine skin layers–heat separated epidermis (HSE), dermatomed and full thickness skin (DS and FTS, respectively) on the stability of a therapeutic peptide. Porcine skin is often used for in vitro permeation studies and is recognized as a good model for human skin (Sekkat et al., 2002; Jacobi et al., 2007). Triptorelin is a luteinizing hormone releasing hormone (LHRH) superagonist currently administered by subcutaneous and intramuscular injection for the treatment of prostate cancer, endometriosis, precocious puberty and female infertility (Sweetman, 2003) and there are several reports on its transdermal delivery using physical and chemical enhancement (Nicoli et al., 2001a,b; Schuetz et al., 2005b). Schuetz et al. reported on the stability of triptorelin in contact with HSE and found that ~10% of the peptide was degraded in 8 h (Schuetz et al., 2005b). However, there are no comparative reports on triptorelin stability in contact with DS and FTS, which are frequently used skin models for evaluating transdermal delivery kinetics in vitro. In the present investigation, we have compared the stability of triptorelin dissolved in pH 7.4 PBS when placed in contact with HSE, DS and FS over a period of 24 h.

Triptorelin acetate was purchased from CBS Technology (Hefei, China), ortho-phosphoric acid and triethylamine were purchased from Acros Organics (Chemie Brunschwig, Basel, Switzerland). All other chemicals used were of at least analytical grade. De-ionized water (resistivity > 18 MOhm cm) was used to prepare all solutions.

A LPG-3400A pump equipped with an ACC-3000 autosampler and a UVD170U UV/vis detector (Dionex; Olten, Switzerland) set at 220 nm was used for quantification of triptorelin. Isocratic separation was performed using a 150 mm × 4.6 mm base stable (from pH 2.0–10) Lichrospher® column packed with 5 μm C18 silica reversed-phase particles and thermostated at 35 °C, and equipped with a prefilter. The mobile phase consisted of 25% acetonitrile and 75% triethylaminephosphate buffer solution (pH 2.3) delivered at a flow rate of 1.5 ml/min (Schuetz et al., 2005b). The injection volume was 100 μl. The column was equilibrated for at least 1 h. All solvents were filtered (0.45 μm nylon membrane filter) and degassed prior to use. Triptorelin eluted at ~2.75 min and the method showed linearity over a concentration range of 1 to 40 μg/ml. The limits of detection and quantification for triptorelin were 0.1 and 0.3 μg/ml, respectively.

Porcine ears were obtained from the local abattoir (CARRE; Rolle, Switzerland) shortly after sacrifice. After cleaning under cold running water, the whole skin was removed carefully from the

* Corresponding author at: Laboratory of Medicinal Chemistry, School of Pharmaceutical Sciences, University of Geneva, 30 Quai Ernest Ansermet, 1211 Geneva, Switzerland. Tel.: +41 22 379 3355; fax: +41 22 379 3360.
E-mail address: yogi.kalia@unige.ch (Y.N. Kalia).

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outer region of the ear and separated from the underlying cartilage with a scalpel. Dermatomed skin (DS) samples with a thickness of ~0.75 mm were prepared using a Zimmer air dermatome (Münsingen, Switzerland). Samples of porcine epidermis were prepared by immersing pieces of fresh full thickness skin in water at 60 °C for 2 min after which the epidermis was carefully separated from the dermis. The different skin samples were wrapped in Parafilm™ and stored at −20 °C; samples were used within 2 months of harvesting.

Triptorelin stability in contact with epidermal and dermal tissues was assessed using standard Franz diffusion cells (contact area $2.0 \pm 0.1\ cm^2$). Skin samples (HSE, DS and FTS) were placed with the interior skin surface facing upward (towards the donor chamber) in all the experiments. A triptorelin solution (3 ml of 0.39 mg/ml peptide in pH 7.4 PBS) was placed in the donor compartment and stirred continuously. Aliquots (0.4 ml) were withdrawn from the donor compartment at hourly intervals for the first 6 h and then a final sample was taken after 24 h. The samples were analyzed using the HPLC method described above.

Fig. 1 shows the time course of triptorelin degradation when placed in contact with HSE, DS and FTS. The data clearly show that triptorelin degradation was extremely rapid when the peptide was in the presence of full thickness skin (FTS). After 3 h, no intact triptorelin was detected in any of the samples; thus, triptorelin had been completely degraded. At the 3 h time-point, $64.9 \pm 8.8\%$ of the peptide in contact with DS had also been modified; after 24 h, the fraction degraded had increased to $87.8 \pm 4.4\%$. Least triptorelin degradation was observed when the peptide was in contact with HSE; after 3 and 24 h the fraction of peptide that had been degraded corresponded to 15.0 ± 4.1 and $51.3 \pm 6.0\%$, respectively, of the applied amount. In control experiments, triptorelin showed good stability in PBS (pH 7.4); the percentage of intact protein at the 6 and 24 h time-points was 98.0 ± 1.4 and $95.0 \pm 0.9\%$, respectively.

Fig. 2 shows a chromatogram from a triptorelin sample in contact with HSE for 1 h-triptorelin eluted at 2.75 min. Three additional peaks (1, 2 and 3) were also observed; they eluted at 1.45, 1.6 and 3.69 min respectively. Similar profiles were obtained at the same time-point (1 h) when triptorelin was placed in contact with DS and FTS (Figs. 3 and 4). An earlier report investigating the solution stability of triptorelin stored in 0.01 M citric acid/phosphate buffer (pH 8.0; 50 °C) for 28 days described the presence of two peaks eluting at a shorter retention time than triptorelin and a third that eluted after the triptorelin peak (Helm and Muller, 1990). The third peak was identified as the free acid, formed by hydrolysis of the C-terminal amide. Solution stability studies with nafarelin, another LHRH superagonist with an amidated C-terminal Gly residue at position 10, have also shown the presence of the free acid derivative as a major degradation product, with a retention time slightly longer than that of the parent molecule (Johnson et al., 1986). Thus,

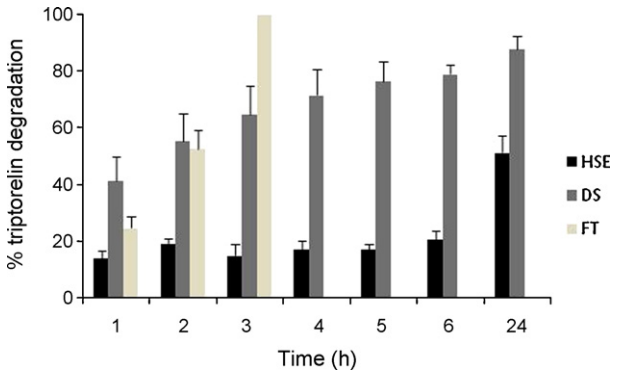

Fig. 1. Triptorelin degradation kinetics when placed in contact with heat separated epidermis (HSE), dermatomed and full thickness skin (DS and FTS, respectively).

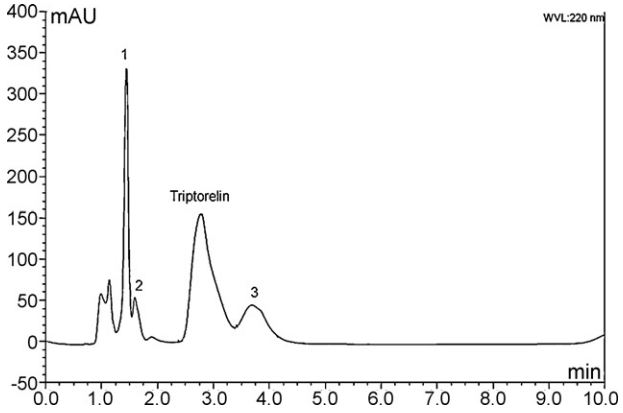

Fig. 2. HPLC chromatogram of triptorelin solution in contact with heat separated epidermis (HSE) after 1 h. Triptorelin elutes at 2.75 min. Additional peaks at 1.45, 1.60 and 3.69 min are clearly observed. Peak 3 at 3.69 min is due to the free acid.

peak 3 in the triptorelin chromatograms (Figs. 2–4) can most likely be attributed to the free acid.

The peaks at shorter retention times in the triptorelin chromatograms were initially thought to be due to fragments generated by the action of endopeptidases. Earlier studies with LHRH showed that it was degraded during iontophoresis, across hairless mouse skin, with formation of the LHRH (1–3) fragment with a retention time of 3.35 min (cf. LHRH which appeared at 7.1 min; Miller et al., 1990). However, iontophoresis under similar conditions of deslorelin, which shares considerable sequence homology with triptorelin,

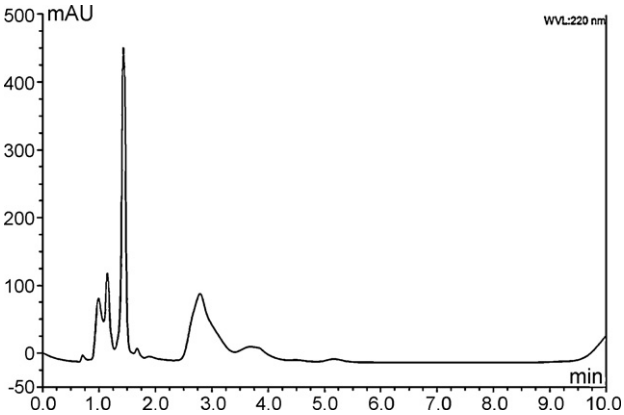

Fig. 3. HPLC chromatogram of triptorelin solution in contact with dermatomed skin (DS) after 1 h.

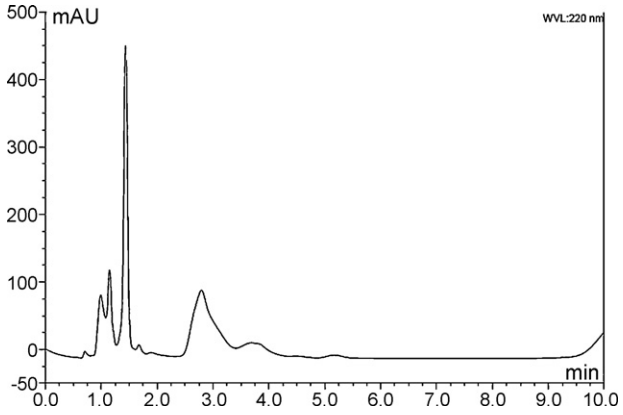

Fig. 4. HPLC chromatogram of triptorelin solution in contact with full thickness skin (FTS) after 1 h.

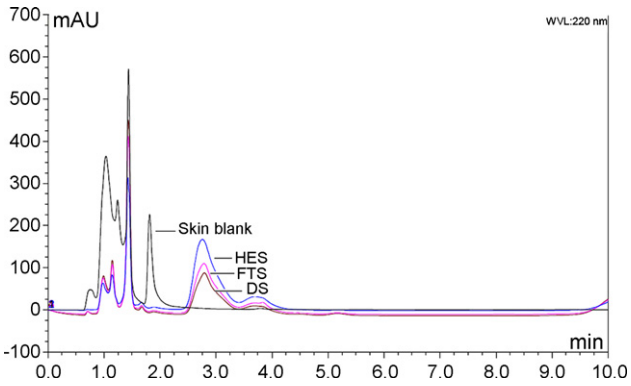

Fig. 5. Superposition of chromatograms of triptorelin solution in contact with HES, DS and FTS (after 1 h) and a control using skin alone. This was used to confirm that peaks 1 and 2 (and the peaks at ~1.0 min) were due to endogenous substances present in the skin.

did not reveal the presence of the Des (1–3) fragment; although two metabolites eluting more quickly than the parent molecule were observed (Miller et al., 1990). However, comparison of the triptorelin chromatograms with that obtained upon placing FTS in contact with buffer for 1 h showed that peaks 1 and 2 (and the split peak observed at $t = 1$ min) were due to substances released from the skin itself (Fig. 5). Although the accelerated stability study described above reported the presence of two peaks eluting at shorter retention times than triptorelin (Helm and Muller, 1990), the conditions employed were considerably more aggressive than those used in the present investigation.

In conclusion, triptorelin was shown to be susceptible to significant degradation when placed in contact with the interior surface of HSE, DS and FTS for periods of up to 24 h. The degradation product appeared to be the free acid formed by hydrolysis of the C-terminal amide. For a given time-point, least degradation was observed in peptide samples placed in contact with the interior surface of HSE-maximum degradation occurred with FTS. This is significant since after traversing the epidermis in vivo the peptide would have ample opportunity to enter the capillary network situated close to the epidermal-dermal interface; thus, decreasing the risk of degradation which seems to be more prevalent in the dermis.

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