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Percutaneous absorption of nafarelin acetate, an LHRH analog, through human cadaver skin and monkey skin

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Abstract

The in vitro skin metabolism and skin permeability of nafarelin acetate, an LHRH analog, through human cadaver skin were investigated. No appreciable metabolic degradation of nafarelin acetate in skin homogenate at pH 5.0 was observed. On the other hand, at pH 7.4, the metabolism of nafarelin acetate by skin homogenate was observed, indicating that skin metabolism of nafarelin acetate is pH dependent. The solubilities of nafarelin acetate in the polyhydroxylated vehicles were several fold higher than those in the monohydroxylated vehicles. The permeability of nafarelin acetate through cadaver skin from various vehicle formulations was evaluated using modified Franz diffusion cells. The skin flux of nafarelin acetate from a propylene glycol (PG)/Azone® vehicle was 0.14 $\mu g/cm^2$ per h. When glyceryl monooleate (GMO) was incorporated into the PG/Azone vehicle mixture, the flux increased by a factor of 1.6. The flux of nafarelin acetate was also determined from a formulation containing ethanol/Azone/GMO (8:1:1). At several fold higher drug concentration, only a 2-fold higher flux was observed compared to the PG/Azone/GMO (8:1:1) formulation. The steady-state lag time ranged from 24 to 40 h. The flux of nafarelin acetate from two vehicle formulations through monkey skin and human cadaver skin was compared. Monkey skin was slightly more permeable than human cadaver skin.

Key words: Nafarelin acetate; Percutaneous absorption; Skin metabolism; Skin flux; Cadaver skin; Monkey skin; Azone®; Penetration enhancer; Solubility

1. Introduction

The systemic administration of biologically active macromolecules such as peptides and proteins to achieve a desired therapeutic effect has always been a challenge to pharmaceutical scientists. This is partly because of the chemical instability of a peptide or protein in a dosage form and partly because of metabolic degradation at the site of administration, especially during oral administration of these drugs due to enzymatic degradation in the gastrointestinal tract. Therefore, recent research has been dedicated to peptide and protein delivery via non-oral routes such as dermal implant, controlled release injectable, intranasal, and transdermal delivery, to circumvent a significant first-pass effect, metabolism at the site of absorption, and to maintain a thera-

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peutic blood level for a longer period of time. Among these various non-oral routes of administration, the transdermal delivery of peptide appears to be an attractive route because it can provide higher systemic bioavailability with negligible first-pass effect and metabolism at the absorption site, skin, as compared to the oral route. Additionally, controlled and sustained delivery of peptide can be accomplished by a transdermal route that would eventually eliminate frequent dosing.

Although the transdermal delivery of peptide and protein emerges as an appealing route of administration, the percutaneous absorption of peptides and small proteins by passive diffusion is limited because of their high molar volume and relatively high hydrophilicity. Hermann et al. (1988) reported that, in contrast to oral administration, topical cyclosporin (cyclic oligopeptide, Mol. Wt = 1201) was not effective in the treatment of psoriasis. This may be partly because of the drug's inability to penetrate the stratum corneum or partly because of the lack of skin metabolism of cyclosporin to active metabolites (Hermann et al., 1988). In contrast, Duncan et al. (1990) demonstrated the in vitro permeability of cyclosporin through cadaver skin from a 5% drug solution in propylene glycol/Azone® (a penetration enhancer). A 6-fold increase in cyclosporin's skin flux was observed when a skin penetration enhancer was incorporated into alcohol and vegetable oil (Duncan et al., 1990). The transdermal delivery of vasopressin (nanopeptide) through excised rat skin and leuprolide (decapeptide, an LHRH analog) through human cadaver skin has been reported (Banerjee and Ritschel, 1989; Lu et al., 1992). These studies demonstrated the transdermal feasibility of a few peptides and small proteins, and this inspired us to evaluate the permeation through human cadaver skin of nafarelin acetate, a decapeptide and an extremely potent LHRH analog. The chemical structure of nafarelin acetate is shown in Fig. 1.

Nafarelin acetate is used clinically for the treatment of endometriosis and is administered intranasally to achieve systemic efficacy (Chrisp and Goa, 1990). Following intranasal administration, peak plasma levels are achieved in 5–40 min, with a terminal half-life of 2.7 h (Hanzel and Monroe, 1988). The daily recommended intranasal dose is 200 μg every 12 h or 400 μg/day. Bioavailability studies indicated that only 2–4% of an applied intranasal dose was bioavailable when compared with an i.v. bolus dose of 25 μg (Chrisp and Goa, 1990). Therefore, only 8–16 μg of nafarelin acetate per day, which is adequate for the prevention of endometriosis in women, reaches the systemic circulation. Transdermal delivery may be an alternative route of administering such a decapeptide for maintaining steady-state therapeutic plasma levels for an extended period of time, as compared to twice-a-day intranasal administration. Moreover, transdermal delivery may further improve the bioavailability of nafarelin acetate because of practically no or little first-pass effect. In the present study, various potential skin penetration enhancers were evaluated to enhance the skin permeation of nafarelin acetate through human cadaver skin. Because nafarelin acetate is a relatively hydrophilic

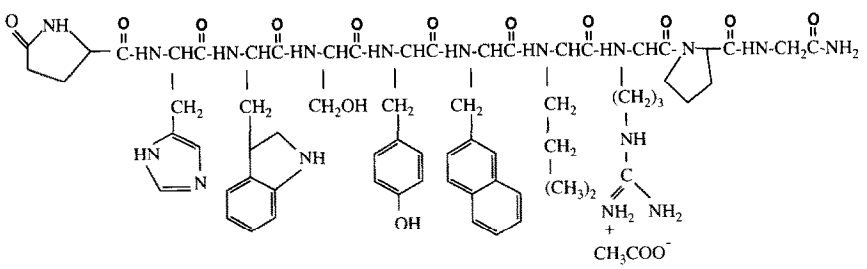

Fig. 1. Chemical structure of nafarelin acetate.

compound, only vehicle mixtures that had a potential to enhance the skin permeability of hydrophilic compounds were selected for vehicle formulations. In addition, our study evaluated the skin metabolism of nafarelin acetate by human cadaver skin homogenates as well as the comparative in vitro permeabilities of nafarelin acetate through monkey and human cadaver skins.

2. Experimental

2.1. Materials

Nafarelin acetate was supplied by the Institute of Organic Chemistry at Syntex (Palo Alto, CA). Azone® was obtained from Nelson Research (Irvine, CA). Transcutol and 2-*n*-nonyl-1,3-dioxolane (Sepa-0009) were a sample gift from Gattefossé (Elmsford, NY) and MacroChem Co. (Billerica, MA). Propylene glycol (PG), absolute ethanol, isopropyl alcohol, glyceryl dioleate, glyceryl monooleate (GMO), lauric acid, and glyceryl monocaprylate were purchased from Sigma (St. Louis, MO) and Aldrich Chemicals (Milwaukee, WI). β-Hydroxypropyl cyclodextrin (βCD) was obtained from Pharmatec Inc. (Alachua, FL). All other chemicals were reagent grade and were used as such without further purification.

2.2. Skin metabolism

Human cadaver skin was used for the metabolism studies. Samples of skin were removed from the inner thigh of human cadaver skin within 24–48 h postmortem with a dermatome set at 300 μm. The split-thickness skin was placed immediately in Ringer's solution and stored at 4°C until further use for skin metabolism and skin permeation studies. Split-thickness skin was cut into small pieces and placed in a porcelain mortar. Liquid nitrogen was added to the skin and the brittle masses were ground with a pestle to a powder. Liquid nitrogen was added periodically as it evaporated. The powdered skin was weighed and 0.1 M KH_2PO_4 buffer (pH 5.0) was added to make an 8% skin homogenate. Skin homogenate was centrifuged at $5000 \times g$ at 4°C for 30 min. The supernatant layer was separated and used immediately for the skin metabolism studies.

The enzymatic reaction was initiated by adding 3 ml of drug solution to 3 ml of skin homogenate that was previously equilibrated to 37°C. The samples (0.5 ml each) were withdrawn from a test tube at the following intervals: 0 (immediately after mixing), 10, 20, 40, 60, and 120 min, and then transferred to another test tube containing 0.5 ml of chilled methanol to arrest the enzymatic reaction instantaneously. The solution was centrifuged at $5000 \times g$ at 4°C for 5 min to remove the precipitated skin homogenate proteins from the supernatant. The supernatant was transferred to a small vial and the samples were assayed by HPLC. A blank sample containing a mixture of skin homogenate and phosphate buffer (1:1 ratio) was incubated simultaneously to ensure the absence of any interfering peaks in the HPLC chromatographs.

2.3. Adsorption studies

Adsorption of nafarelin acetate by various diffusion cells was studied. Three types of diffusion cells composed of acrylate, Teflon®, and glass materials were selected for adsorption studies. The glass cells were silanized with 2% dichlorodimethylsiloxane in toluene to prevent significant adsorption of nafarelin acetate. Briefly, a known amount of nafarelin acetate solution in a phosphate buffer (pH 5.0) was added to each diffusion cell and incubated for 3 days at 32°C. A sample (1 ml) from each diffusion cell was withdrawn at the end of 3 days and the samples were assayed by HPLC. The amount of the drug adsorbed by each diffusion cell was determined by subtracting the initial from the final drug concentration remaining after 3 days of incubation.

2.4. Solubility determinations

The solubilities of nafarelin acetate in various pharmaceutical vehicles were determined by equilibrating large excesses of the solute and the vehicle in a small glass vial. The glass vial was placed in a water bath maintained at 32°C and

the solution was stirred continuously for 48 h to reach equilibrium. Samples (1 ml) were withdrawn, filtered through glass-wool-tipped pipets, and an appropriate volume of the filtrate was diluted with methanol prior to HPLC assay.

2.5. Skin permeation procedure

Modified Franz diffusion cells (acrylic type) were used for the skin permeation studies. The split-thickness cadaver skin was mounted between two halves of a Franz diffusion cell and the entire cell was immersed in a water bath maintained at 32°C. The receiver compartment was filled with phosphate buffer of pH 5.2 containing 0.1% NaN_3 as an antimicrobial agent. The receiver fluid was stirred continuously with a magnetic bar at a speed of 300 rpm. The receiver fluid was equilibrated for at least 1 h. Skin permeation was initiated by charging the donor compartment with a drug solution. At predetermined intervals, 0.7 ml of sample from the receiver compartment was withdrawn and immediately replaced with previously warmed 0.7 ml of the same buffer solution. The samples were assayed by HPLC.

The in vitro skin permeation of nafarelin acetate through human cadaver skin and monkey skin was compared. Monkey skin was harvested from the abdomen of freshly killed *Cynomolgus* monkeys. The fat was removed from the full-thickness skin with the aid of scalpels and scissors, and the skin was either used immediately for skin permeation studies as described or stored at −20°C for later studies.

2.6. Drug assay

Nafarelin acetate was assayed by HPLC with UV detection. The Vydac protein and peptide column (C_{18}, 300 Å pore size, 5 μm, and 250 × 4.6 mm) in conjunction with a precolumn was used for chromatographic resolution of nafarelin acetate. The mobile phase was 30% acetonitrile/70% KH_2PO_4 (0.175 M). The other chromatographic conditions were as follows: flow rate, 1 ml/min; $\lambda_{max} = 237$ nm. The peak area was integrated by a Spectra Physics 4700 Integrator.

2.7. Data analysis

The skin flux was determined from Fick's law of diffusion:

$$dM/dt = J_s = P_e \Delta C \quad (1)$$

where M is the cumulative amount of drug permeated through the skin as a function of time t, J_s denotes the skin flux in μg/cm² per h, P_e is the effective permeability coefficient (cm/h), and ΔC represents the concentration gradient across the skin, which is assumed to be the same as the donor concentration provided that the receiver concentration never exceeds 10% of the donor.

3. Results and discussion

3.1. Physicochemical properties and solubilities of nafarelin acetate

The physicochemical properties of nafarelin acetate are summarized in Table 1. Three out of 10 amino acid residues in nafarelin are ionizable: histidine, tyrosine, and arginine. The pK_{a_1} and pK_{a_2} values of nafarelin acetate have been reported to be 6.05 (histidine) and 9.92 (tyrosine) (Nestor et al., 1982). Based on the isolated amino acid, the pK_{a_3} for the arginine moiety in nafarelin was estimated to be 12.5. It is interesting that nafarelin acetate exists as a di-cation at pH < 5.5, but the drug exists as a mono-cation at pH 6.5–9.5.

Table 1
Physicochemical properties of nafarelin acetate [a]

Mol. Wt (g/mol)	1384.5 (anhydrous monoacetate)
Melting point (°C)	185–193
pK_{a_1}	6.05 (histidine)
pK_{a_2}	9.92 (tyrosine)
$K_{octanol/water}$	0.007 at pH 4.0; 0.3 at pH 7.4
Solubility in phosphate buffer (pH 7.6) at 25°C (mg/ml)	0.97
λ_{max} (nm)	225

[a] Nestor et al. (1982).

On the other hand, at pH greater than 10.5, nafarelin exists as a zwitterion. Because of varying degrees of ionization of nafarelin at different hydrogen ion concentrations, the octanol/water partition coefficient ($K_{octanol/water}$) of nafarelin acetate is expected to be aqueous pH dependent. Indeed, the $K_{octanol/water}$ of nafarelin increased from 0.007 to 0.3 as the pH of the aqueous solution was increased from 4.0 to 7.4 (Table 1). This is not surprising because nafarelin acetate existed as a mono-cation at a higher pH (a less water soluble species) as opposed to a di-cation at a lower pH (a more water soluble species).

The solubility of nafarelin acetate in the phosphate buffer at pH 7.6 was about 1 mg/ml. A higher aqueous solubility was observed at a lower pH (< 5.5); however, upon standing, the drug aggregated to form a clear viscous gel. The rate of formation of a gel appeared to be dependent on the degree agitation/foaming and the degree of supersaturation. Table 2 summarizes the solubility of nafarelin acetate in selected polar and non-polar pharmaceutical vehicles. The drug's solubilities in the polyhydroxylated vehicles such as propylene glycol (PG), transcutol, and glyceryl mono-dicaprylate were significantly higher than those in the monohydroxylated vehicles of absolute ethanol and isopropyl alcohol. The higher solubility of nafarelin acetate in polyhydroxylated vehicles was possibly because of increased interaction between the polar functional groups of nafarelin and hydroxyl and/or carboxyl moieties of the vehicles studied. It is rather surprising that the solubility of nafarelin acetate in glyceryl dioleate was significantly higher than that in absolute ethanol and isopropyl alcohol, even though the former vehicle was relatively less hydrophilic than the latter two. It is suspected that the commercial glyceryl dioleate may contain traces of polar components such as glycerol, glyceryl monooleate, and oleic acid, which might have contributed to the higher solubility of nafarelin acetate. Nafarelin acetate formed a firm gel in the polyhydroxylated vehicles due to its higher solubility, while a clear solution of nafarelin acetate was obtained in absolute ethanol and isopropyl alcohol because of its low solubility in these vehicles. It appeared that nafarelin acetate had a tendency to form a gel in those vehicles, both aqueous and non-aqueous media, where it demonstrated a higher drug solubility.

Table 2
Solubilities of nafarelin acetate in various pharmaceutical vehicles at 32°C

Vehicle	Solubility (mg/ml)
Isopropyl alcohol	0.6
Absolute ethanol	3.0
Glyceryl dioleate	160.0
Propylene glycol	210.0
Transcutol	288.0
Glyceryl monocaprylate	308.0

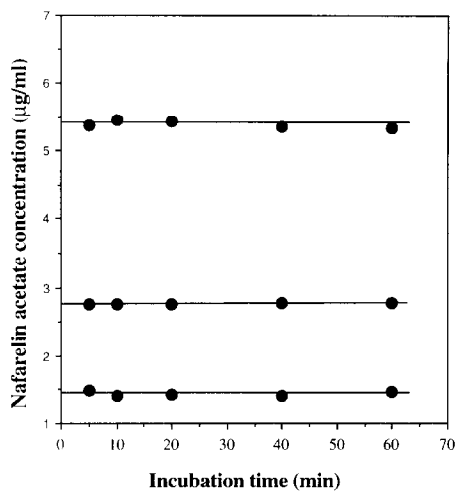

Fig. 2. In vitro metabolism of nafarelin acetate by human cadaver skin homogenate at pH 5.0 at 37°C.

3.2. Skin metabolism of nafarelin acetate

The concentration of nafarelin in the skin homogenate at pH 5.0 as a function of time is shown in Fig. 2. The skin metabolism of nafarelin acetate was studied at three concentration levels. No appreciable metabolic degradation of nafarelin acetate at pH 5.0 upon incubation of the drug with the skin homogenate was observed. It thus appeared that cadaver skin had little or no peptidase activity to metabolize nafarelin acetate at pH 5.0.

Fig. 3 shows the skin metabolism of nafarelin acetate at pH 7.4. The concentration of nafarelin

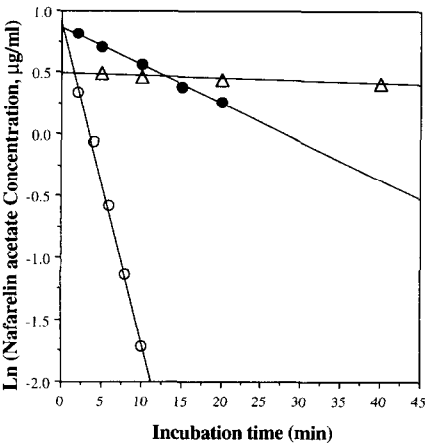

Fig. 3. In vitro metabolic degradation of nafarelin acetate at 32°C. Trypsin (○); skin homogenate at pH 7.4 (●); phosphate buffer at pH 7.4 (△).

acetate in the skin homogenate declined exponentially as a function of time, while no appreciable change in the drug concentration in the phosphate buffer solution at the same pH was observed. When nafarelin acetate was incubated with 0.1 M trypsin solution at pH 7.0, the metabolic degradation of nafarelin acetate was several times faster than the cadaver skin homogenate, thus validating the techniques of measurement. These results suggest that the in vitro skin metabolism of nafarelin acetate was pH dependent, with a high metabolic rate at physiological pH.

3.3. Skin permeation of nafarelin acetate

Table 3 summarizes the adsorption of nafarelin acetate by various diffusion cells after 3 days of incubation. At 0.1 µg/ml concentration, the adsorption of nafarelin acetate by the acrylate diffusion cells was minimal compared to Teflon® and silanized-glass diffusion cells. At the 1.0 µg/ml concentration, the acrylate and silanized-glass diffusion cells showed very little adsorption of nafarelin acetate. In contrast, only 72% of the initial drug concentration remained in the Teflon diffusion cells because of adsorption of nafarelin acetate. These results clearly suggested that the acrylate and silanized-glass diffusion cells were

Table 3
Adsorption of nafarelin acetate by various diffusion cells after 3 days

Drug concentration (µg/ml)	Diffusion cell material	% original strength after 3 days at 32°C
0.1	acrylate	82 ± 4
	teflon	72 ± 2
	silanized glass	76 (72, 79)
1.0	acrylate	90 ± 7
	teflon	72 ± 12
	silanized glass	95 (96, 94)

the most suitable material for skin permeation experiments since they adsorbed the least amount of nafarelin acetate among the three cells studied. In the present study, however, we used the acrylate diffusion cells for convenience as the glass diffusion cells required a silanization step prior to use.

Fig. 4 shows the representative cumulative amount-time profiles of nafarelin acetate from the two transdermal vehicle formulations. It is interesting that no significant amount of nafarelin acetate in the receiver compartment was detected during the first 20 h of diffusion experiments. A steady-state skin flux was attained after 24 h and maintained for the duration of the diffusion studies; the experiments were terminated after 4 days to ensure the integrity of the stratum corneum.

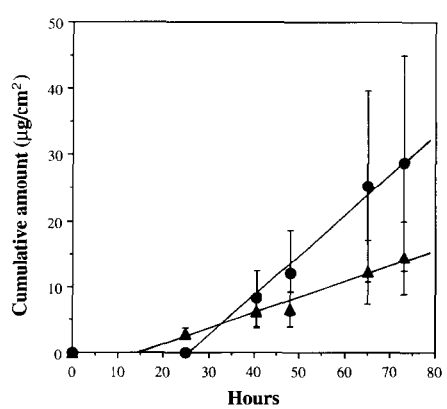

Fig. 4. Representative permeation profiles of nafarelin acetate through human cadaver skin from two vehicle formulations. PG/Azone/GMO (●); ethanol/Azone/GMO (▲).

Table 4 summarizes the permeation parameters of nafarelin acetate from the various vehicle formulations. The steady-state skin flux was determined from the linear portion of the cumulative amount vs time plot; the lag time was estimated by extrapolating the steady-state slope to the abscissa. When PG was used as a vehicle, no detectable amount of nafarelin acetate was observed in the receiver compartment after 3 days of skin permeation study (data not shown). On the other hand, when 10% Azone was incorporated into PG, a steady-state skin flux of 0.14 $\mu g/cm^2$ per h was observed. This implied that Azone had the ability to modify the permeability of the stratum corneum, leading to a relatively higher skin flux of nafarelin acetate.

When GMO was incorporated into the PG/Azone vehicle mixture, the skin flux was increased by a factor of 1.6 compared to that of the PG/Azone vehicle alone. Both PG/Azone and PG/Azone/GMO vehicles formed viscous gel at a high drug concentration (> 70 mg/ml). As a result, the thermodynamic activity of nafarelin acetate in the gel would be significantly lower than those vehicles that displayed a clear solution. Such a decrease in a chemical driving force would lead to a decrease in the skin flux of nafarelin acetate. Under this assumption, selection of an appropriate vehicle (with low drug solubility) and incorporation of an anti-gelling agent in a vehicle to circumvent the gel formation would eventually lead to a high skin flux of nafarelin acetate. Therefore, 50% PG was replaced with 50% absolute ethanol to suppress the drug solubility; β-hydroxycyclodextrin (βCD) was added to the PG/Azone vehicle mixture to circumvent gel formation (Table 4). Indeed, the PG/ethanol/Azone/βCD (10:8:1:1) formulation provided a clear solution even at saturation (48.1 mg/ml), and the skin flux of nafarelin acetate from such a vehicle system was comparable to that of the PG/Azone/GMO (8:1:1) vehicle system. Interestingly, when 5% lauric acid was added to the PG/ethanol (9:10) vehicle mixture, the skin flux of nafarelin acetate was 0.27 $\mu g/cm^2$ per h, virtually the same as that of the PG/Azone/GMO vehicle system. In contrast, when 10% Sepa-0009 was added to the PG/ethanol (1:1) vehicle, nafarelin acetate skin flux was significantly lower than that of the PG/Azone formulation, even though no obvious gel formation in the former vehicle was observed. These results suggested that, in addition to high thermodynamic activity of the drug in the solution (i.e., no gel formation), the skin penetration enhancer was essential to accelerate the skin flux of nafarelin acetate through human cadaver skin. In these studies, however, we did not evaluate the influence of intramolecular hydrogen bonding between nafarelin acetate and the polar functional group of a vehicle on the skin flux of nafarelin acetate. It is generally believed that intramolecular hydrogen bonding would substantially reduce the membrane transport of peptide and protein (Burton et al., 1991).

It is well documented that PG is an optimum vehicle for Azone to enhance the skin flux of a majority of hydrophilic drugs (Wotton et al., 1985;

Table 4
Skin permeation parameters of nafarelin acetate from various transdermal formulations

Vehicle composition (w/w)	Drug concentration (mg/ml)	J_s ($\mu g/cm^2$ per h)	T_{lag} (h)	ER [a]
PG/Azone (9:1)	100.0 [b]	0.14 ± 0.08	30	1.0
PG/Azone/GMO (8:1:1)	114.0 [b]	0.23 (0.33, 0.13)	21	1.6
PG/ET/Azone/βCD (10:8:1:1)	48.1 [c]	0.22 ± 0.11	30	1.6
PG/ET/Lauric acid (9:10:1)	47.7 [c]	0.27 (0.3, 0.23)	41	1.9
PG/ET/Sepa-0900 (9:9:2)	49.4 [b]	0.09 ± 0.05	10	0.6

PG, propylene glycol; GMO, glyceryl monooleate; ET, ethanol; βCD, β-hydroxypropylcyclodextrin. All data were obtained from the single skin donor to avoid any intersubject variability in skin flux.

[a] ER, enhancement ratio = skin flux from a vehicle mixture/skin flux from PG/Azone vehicle.

[b] Formed gel.

[c] Clear solution.

Table 5
Permeation of nafarelin acetate through cadaver skin from transdermal formulations

Vehicle composition (w/w)	Drug concentration (mg/ml)	J_s ($\mu g/cm^2$ per h)	$P_e(\times 10^6)$ (cm/h)	T_{lag} (h)
PG/Azone/GMO (8:1:1)	177.1 [a]	0.54 (0.63, 0.44)	3.0	32
ET/Azone/GMO (8:1:1)	12.0 [b]	0.25 ± 0.13	21.0	11
PG/ET/Azone/βCD (10:8:1:1)	44.2 [b]	0.60 ± 0.09	14.0	37

PG, propylene glycol; GMO, glyceryl monooleate; ET, ethanol; βCD, β-hydroxypropylcyclodextrin. All data were obtained from the same skin donor to avoid any intersubject variability in skin flux.
[a] Formed gel.
[b] Saturated solution but no gel.
[c] Unsaturated solution.

Sheth et al., 1986; Banerjee and Ritschel, 1989). Because the solubility of nafarelin acetate in PG was several times higher than that in absolute ethanol, the skin flux of nafarelin acetate from the two different vehicle systems containing Azone/GMO (1:1) was evaluated. The skin flux of nafarelin acetate through human cadaver skin from the three vehicle formulations is summarized in Table 5. Although the concentration of nafarelin acetate in PG/Azone/GMO (8:1:1) vehicle was several times higher than that of the ethanol/Azone/GMO (8:1:1) vehicle, only a 2-fold increase in the skin flux of nafarelin acetate from the former vehicle system was observed. The effective permeability coefficient ($P_e = J_s/\Delta C$) of nafarelin acetate in these two vehicles was compared. The P_e of nafarelin acetate in the ethanol/Azone/GMO vehicle mixture was about 7-fold higher than that of the PG/Azone/GMO vehicle mixture. Furthermore, the lag time ($T_{lag} \propto 1/D_{sc}$, where D_{sc} is the diffusion coefficient of the drug in the stratum corneum) of nafarelin acetate in the ethanol/Azone/GMO vehicle was

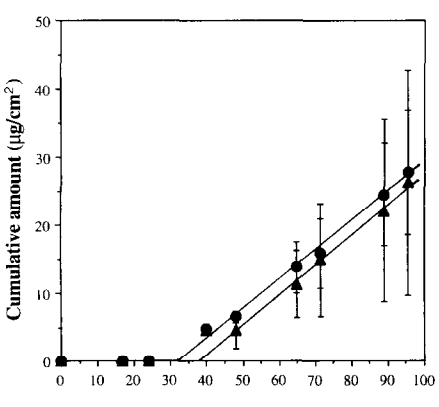

Fig. 5. Representative permeation profiles of nafarelin acetate through monkey skin from two vehicle formulations. PG/Azone/GMO (●); PG/ethanol/Azone/βCD (▲).

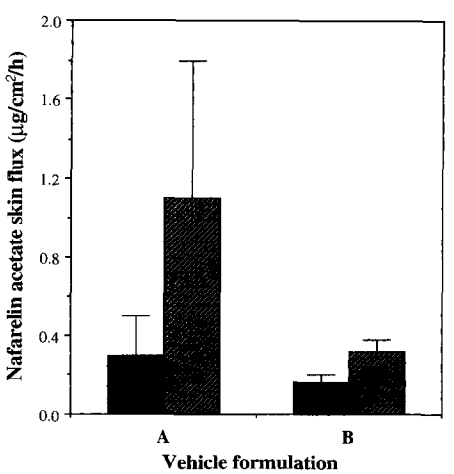

Fig. 6. Comparative skin flux of nafarelin acetate from two vehicle formulations. Human cadaver skin (closed bar); monkey skin (hatched bar); (A) PG/ethanol/Azone/βCD; (B) PG/GMO/Azone; nafarelin acetate concentration, 56 mg/ml; pH of receiver fluid, 7.4.

about 3-times lower than that of the PG/Azone/GMO vehicle mixture. Since P_e is the product of D_{sc} and the skin/vehicle partition coefficient, a 3-fold increase in diffusivity does not explain the 7-fold increase in the P_e of nafarelin acetate. It is possible that an increase in the D_{sc} and a high skin/vehicle partition coefficient of the drug in the presence of absolute ethanol were responsible for the enhanced permeability of nafarelin acetate through cadaver skin. This implied that the addition of absolute ethanol would be desirable to enhance the skin flux of nafarelin acetate. On the other hand, the skin flux of nafarelin acetate from the PG/ethanol/Azone/βCD vehicle was virtually the same as that of the PG/Azone/GMO vehicle, even though the drug concentration in the former vehicle was about 4-times lower than that of the latter vehicle.

The permeability of nafarelin acetate through monkey skin and human cadaver skin was compared. Fig. 5 shows the representative permeation profiles of nafarelin acetate through monkey skin from two vehicle formulations. The permeation profiles of nafarelin acetate in monkey skin were very similar to those of human cadaver skin. Again, no significant amount of nafarelin acetate in the receiver fluid was detected during the first 24 h of diffusion experiments. A steady-state skin flux was attained after 24 h and maintained for the duration of the experiments.

The skin fluxes of nafarelin acetate through human cadaver skin and monkey skin from two vehicle formulations were simultaneously compared to demonstrate species variations. Fig. 6 illustrates the comparative skin fluxes of nafarelin acetate from the two transdermal formulations. The skin flux of nafarelin acetate in monkey skin from PG/ethanol/Azone/β-CD (formulation A) was roughly 2.5-times higher than that in human cadaver skin while only a slight increase in nafarelin's skin flux from PG/GMO/Azone (formulation B) was observed. Although monkey skin was slightly more permeable than human cadaver skin, monkey skin can be successfully used as a substitute for human cadaver skin to rank order nafarelin acetate transdermal formulations.

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