Transdermal Delivery of Levonorgestrel IV: Evaluation of Membranes

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Abstract □ A series of experiments were performed to evaluate the flux of levonorgestrel (LN), ethyl acetate (EtAc), and ethanol (EtOH) through excised rat skin, through a variety of synthetic membranes and through membranes supported on rat skin. Using a donor phase of EtAc:EtOH (7:3) containing excess solid LN, the flux of LN through rat skin was ~1.0 $\mu g/cm^2 \cdot h$. The normalized fluxes of LN, EtAc, and EtOH through ethylene vinyl acetate (EVAc) copolymers of varying vinyl acetate (VAc) content (12 to 25%) were 1.3 to 3.1 $\times 10^{-8}$, 2.6 to 6.8 $\times 10^{-4}$, and 4.8 to 9.9 $\times 10^{-5}$ $g \cdot cm/cm^2 \cdot h$, respectively. Permeability experiments were also performed with the EVAc membranes supported on rat skin. By selecting the VAc content and thickness of the EVAc membranes, it was possible to control the delivery of enhancer (EtAc:EtOH) through rat skin (membrane-rate control) or to let the skin control the overall delivery of enhancer.

The transdermal route for controlled drug delivery is receiving considerable attention. A major problem encountered with many drugs is the low permeability of human skin, as well as its biological variability.[1,2] One way to reduce this problem is to include in the transdermal formulation one or more chemicals which reversibly reduce the barrier properties of the skin, allowing more drug to penetrate into the viable tissues and the systemic circulation.[3–5] Such chemicals are known as permeation enhancers.

A number of chemicals are useful permeation enhancers. Despite the identification of several very effective skin permeation enhancers,[3,5,6] such as dimethyl sulfoxide,[7] Azone (laurocapram; 1-dodecylhexahydro-2*H*-azepin-2-one),[8] dimethylformamide, and dimethylacetamide,[9] their usefulness in commercial transdermal products has yet to be demonstrated. Toxicity and skin irritation have generally limited the practical application of these chemicals in transdermal drug delivery systems. Thus the search continues for safe, effective, and hopefully generically useful permeation enhancers.

During the course of our research to develop a transdermal delivery system for levonorgestrel (LN), we have investigated permeation enhancers[10] and prodrugs[11] to increase the skin permeability of this very potent, yet very lipophilic contraceptive steroid. A number of solvents were investigated for their ability to increase the percutaneous absorption of LN, including ethanol (EtOH), which is an effective permeation enhancer for 17β-estradiol.[12] However, EtOH was not very effective as an enhancer when tested with LN.[10] Another solvent tested as an enhancer for LN, as described in the third paper of this series, was ethyl acetate (EtAc).[13] This common organic solvent was found to be a very effective permeation enhancer for LN and a number of other drugs used with or without EtOH as a cosolvent.[13,14]

Preparing a transdermal delivery system for LN with EtAc as a permeation enhancer places certain constraints on device design. Preliminary experiments indicated that EtAc is very skin permeable and that relatively large amounts of this solvent were required to sufficiently increase skin permeability. We have therefore chosen a reservoir type device to codeliver drug and enhancer(s) to the skin. As such, a synthetic membrane is required to contain the drug:enhancer suspension and, if needed, to control the delivery of drug or enhancer(s) to the skin. This paper describes the evaluation of various membranes which may be useful for a reservoir transdermal device for LN.

Experimental Section

Materials—Ethylene vinyl acetate copolymers (EVAc), high density polyethylene (HD-2), and Hytrel 7246 (polyester elastomer) were obtained from Dupont (Wilmington, DE). Ethylene vinyl acetate (VA 24) was also obtained from CT (Tustin, CA), and polymethylpentene (TPX 845) was obtained from Mitsui (Los Angeles, CA). The medical grade adhesive BIO-PSA X7-2920 was obtained from Dow Corning (Midland, MI).

Levonorgestrel (LN) was a gift from the World Health Organization. Ethyl acetate (EtAc; reagent grade) and hydroxypropyl cellulose (1×10^6 MW) were purchased from Aldrich Chemical (Milwaukee, WI) and were used as received. Ethanol (EtOH; 200 proof, U.S.P. grade) was purchased from USI Chemicals (Tuscola, IL). The rats (male, Wistar strain) were obtained from Simensen Labs (Gilroy, CA).

Permeability Studies with Rat Skin and Membranes—A system employing nine glass Franz diffusion cells was used for the permeability experiments with rat skin and membranes. The Franz cells were modified with inlet and outlet receiver phase ports to allow continuous flow through the cells.

The rats (180–220 g) were sacrificed in a CO_2 chamber, and an approximately 6-cm^2 area of full-thickness skin was excised from the shaved (mechanical clippers) abdominal site. After removal of the subcutaneous fat, the skin was washed with physiological saline and used in the permeability experiment within 1 h. The skin or membrane was mounted and clamped between the cell body and the call cap; the furry side was faced upward (donor side) when using rat skin. For testing permeability of LN, EtAc, and EtOH through membrane/rat skin, the adhesive BIO-PSA X7-2920 was cast onto the membranes at a thickness of 25 μm. These membranes were then sealed to the rat skin in the diffusion cell. The surface area exposed to the donor phase was 5.07 cm^2. The donor phase (~5 mL) was prepared by suspending excess solid drug in the EtAc:EtOH (7:3, v/v). (The compounds EtAc and EtOH are completely miscible in each other.) For experiments performed with membranes supported on rat skin, the donor phase was gelled with 2 wt % hydroxypropyl cellulose. The donor phase suspension was applied directly on the skin or membrane through the cell cap, which was then sealed with a glass stopper. The receptor phase, in contact with the underside of the skin or membrane, was isotonic saline at 37 °C with 0.1% sodium azide added to prevent bacterial growth. The cells were maintained at 37 °C by thermostatically controlled water which was circulated through a jacket surrounding the cell body. The donor phase temperature was measured at 32 °C.

Receiver phase solution was pumped through the diffusion cells by means of a Manostat Cassette Pump drive unit. A fraction collector was used to collect the cell effluent. The flow rate was set so that the drug concentration in the receptor phase remained at <10% of saturation. Uniform mixing of the drug in the receiver phase was

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achieved by a small magnetic stirring bar driven by an external 600 rpm motor. The donor suspensions were changed daily to prevent dilution with water which can enter the donor chamber by osmosis.

Preparation of Membranes—Polymethylpentene (TPX 845), VA 24, and Hytrel 7246 were supplied as membranes at thicknesses of 76, 64, and 25 μm, respectively. All other membranes were prepared by placing the commercial pellets between Teflon-faced aluminum plates and heating to the Vicat softening point.[15] Pressure was applied for 1 min at 30,000 psi and the plates were then cooled. The finished films were peeled from the Teflon surface and inspected. The thickness of each membrane was measured by a Mercer caliper snap gauge, which is accurate down to 2 μm.

Chromatographic Analysis—The LN concentration in the receptor phase was measured using HPLC. No sample pretreatment was required. The HPLC analyses were performed on a Waters 840 system consisting of two model 510 pumps, a model 481 UV detector, a model 710B WISP (sample processor), and a Digital computer model 350 microprocessor/programmer. The column used to separate LN was a 4.6 mm × 25 cm, 10 μm, Whatman ODS-3 Partisil C-18. The LN was measured with a mobile phase of acetonitrile:H_2O (50:50; v/v) at a flow rate of 2.0 mL/min, with absorbance monitoring at 243 nm. The retention time of LN was 6.0 min.

Ethanol (EtOH) and EtAc were measured in the receptor phase with a Waters column (7.8 mm × 15 cm). The mobile phase used was 0.05% H_3PO_4 in H_2O (v/v) at a flow rate of 1.5 mL/min. The compounds EtAc and EtOH were detected with a Waters R-400 differential refractometer. The retention time of EtOH was 4.2 min, while that of EtAc was 6.6 min.

Results

A series of experiments was performed to evaluate the flux of LN, EtAc, and EtOH through excised rat skin, through a variety of synthetic membranes, and through membrane/rat skin systems. The donor phase composition used in most the experiments was a solution of EtAc:EtOH (7:3, v/v) which contained excess solid drug. A cosolvent system of EtAc:EtOH (7:3) was used because the addition of EtOH to EtAc was found to increase the flux of LN to a greater extent than did pure EtAc.[13] Excess solid drug was used to insure a constant, maximum driving force in all formulations.[16]

Flux of Levonorgestrel, Ethyl Acetate, and Ethanol Through Rat Skin—The flux of LN through rat skin from the standard donor phase was found to be ~0.9 to 1.0 μg/cm²·h at steady state. The flux of LN is shown graphically in Figure 1. For comparison, the flux of LN from donor phase solvents of pure water and pure EtOH saturated with excess LN is also shown. The flux enhancement of LN using an EtAc:EtOH (7:3) donor phase relative to water as the donor phase solvent was ~100 fold; relative to EtOH as donor phase solvent, flux enhancement was ~17 fold.

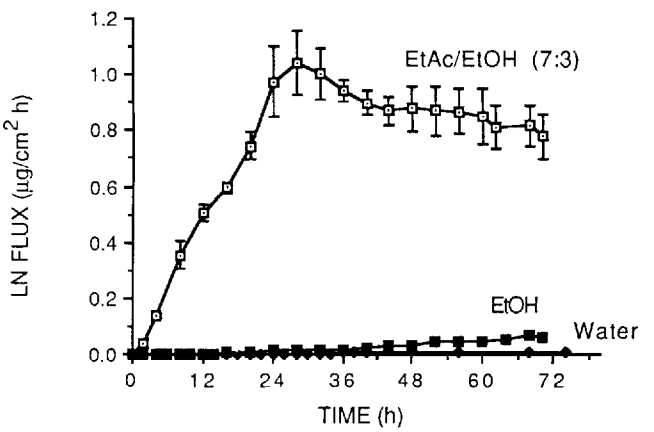

Figure 1—Flux of LN through rat skin in vitro using EtAc:EtOH (7:3, v/v; n = 3), pure EtOH (n = 4), and pure water (n = 3) as donor phase solvents. Error bars are mean standard error.

The flux of EtAc and EtOH through rat skin from the standard donor phase was found to range from 4 to 6 mg/cm²·h and 8 to 10 mg/cm²·h, respectively, at steady state. The flux values for LN, EtAc, and EtOH can be used in conjunction with membrane permeability data to determine whether a device using such a membrane would be rate limiting with regard to drug or enhancer.

Permeability of Membranes Towards Levonorgestrel, Ethyl Acetate, and Ethanol—Several membranes were screened initially to measure the permeation of LN from a donor phase solvent of pure EtOH. These membranes included TPX 845 (polymethylpentene), HD-2 (high-density polyethylene), VA 24 (EVAc, 4 to 6% VAc content], and Hytrel 7246 (polyester elastomer). The permeation of LN through all these membranes was too low to be considered further as a component of a transdermal delivery system for LN.

The polymer membranes investigated next were EVAc membranes with VAc contents ranging from 12 to 25%. Generally, low molecular weight chemicals exhibit increasing permeation through EVAc membranes with increasing VAc content.[17,18] The permeability of EVAc membranes (50 and 100 μm thicknesses) were measured using modified Franz diffusion cells, also used with the rat skins. The membrane was placed between the donor and receptor phase, and the standard donor phase (5 mL) was placed on the membrane. The appearance of LN, EtAc, and EtOH in the receptor phase was measured by HPLC. The normalized fluxes[16] of LN, EtAc, and EtOH through the EVAc membranes tested (12, 15, 18, and 25% VAc) are shown in Table I. The permeability of the EVAc membranes increased as expected with increasing VAc content for all three components. The absolute flux of LN through these membranes (100 μm thick) ranged from 1.8 to 4.0 μg/cm²·h at steady state. The LN flux through an EVAc membrane of 18% VAc content at 50 and 100 μm thicknesses is shown in Figure 2. The flux of LN ~2.5 μg/cm²·h through a 50-μm thick membrane of EVAc (18% VAc) is significantly greater than that through rat skin (1.0 μg/cm²·h). If this membrane were used with a transdermal device, the release of LN would be predominantly skin-rate controlled under the conditions used. The flux of LN through the 100-μm thick membrane of EVAC (18% VAc) is much closer to that of LN through skin. Hence, a transdermal device would be expected to deliver LN with the rate of delivery controlled by both the skin and the membrane. This example uses rat skin; the situation would differ using human skin which is less permeable than is rat skin towards LN.[10] The variations in LN flux over time (see

Table I—Permeability of Ethylene Vinyl Acetate (EVAc) Membranes

Vinyl Acetate (VAc) Content, %	Component[a]	Normalized Flux, g·cm/cm²·h
12	LN	1.3×10^{-8}
	EtAc	2.6×10^{-4}
	EtOH	4.8×10^{-5}
15	LN	1.4×10^{-8}
	EtAc	2.3×10^{-4}
	EtOH	6.9×10^{-5}
18	LN	1.7×10^{-8}
	EtAc	3.5×10^{-4}
	EtOH	7.9×10^{-5}
25	LN	3.1×10^{-8}
	EtAc	6.8×10^{-4}
	EtOH	9.9×10^{-5}

[a] Donor phase used was EtAc:EtOH (7:3, v/v) saturated with excess LN; membrane thicknesses were 50 ± 10 and 100 ± 10 μm.

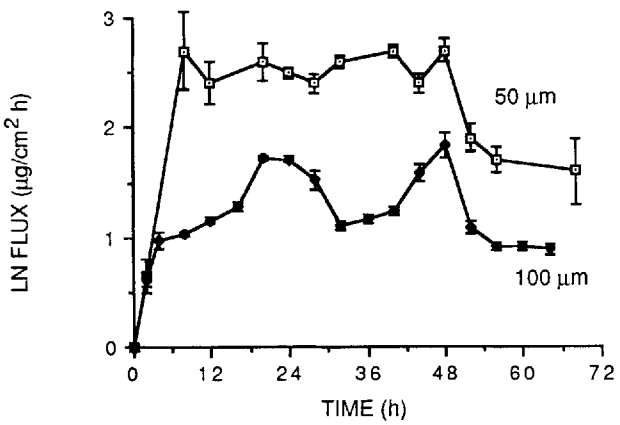

Figure 2—Flux of LN through EVAc membranes (18% VAc content) of 50 ($n = 3$) and 100 μm ($n = 3$) thicknesses. Error bars are mean standard error.

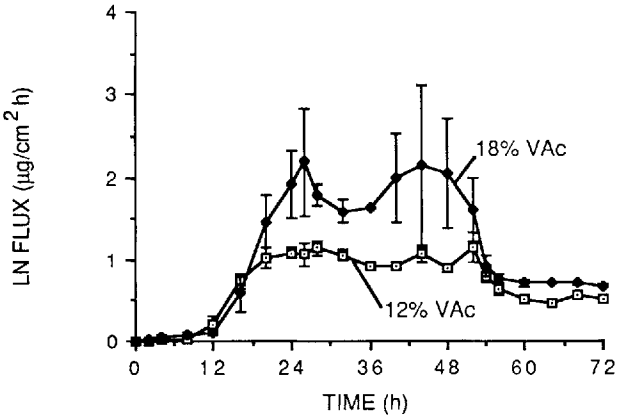

Figure 3—Flux of LN through EVAc membranes (18 and 12% VAc content, 50 μm thick) supported on rat skin. The donor phase was EtAc:EtOH (7:3, v/v) gelled with 2 wt % hydroxypropyl cellulose. Error bars are mean standard error.

Figure 2) are probably due to the replacement of the donor solutions every 24 h.

Flux of Levonorgestrel, Ethyl Acetate, and Ethanol Through Membrane/Rat Skin Systems—The EVAc membranes tested for permeability (12 through 25% VAc content) were also tested with rat skin. These experiments were performed using EVAc membranes (50, 100, or 200 μm thickness) coated on one side with the medical grade adhesive BIO-PSA X7-2920 at a thickness of 25 μm. The membranes were firmly attached to the rat skin and the standard donor phase, which was gelled with 2 wt % hydroxypropyl cellulose, was placed on the membrane. Thus, the drug and enhancers permeated through the membrane, adhesive, and then the rat skin.

The flux data collected from the membrane/rat skin experiments are given in Table II. With the exception of the 12% VAc content EVAc membranes at a 200-μm thickness, the membrane/rat skin tested did not impede the overall flux of LN relative to using rat skin alone. The flux of EtAc and EtOH through rat skin using the two restrictive membranes was lower than through rat skin alone. This indicates that with less enhancer being delivered through the skin, drug flux is lower. The LN flux through the 12% VAc membranes of the thicknesses tested was greater than the overall flux through membrane/rat skin using the same membrane. The other membranes tested did not restrict the delivery of EtAc and EtOH sufficiently to reduce overall LN flux. However, both the 15 and 18% VAc content EVAc membranes at 50 μm thickness did appear to control the delivery of EtAc to the skin. Nonetheless, LN flux was as great or greater than flux of LN through rat skin only using the same enhancer system.

The flux of LN through EVAc membranes (18 and 12% VAc content, 50 μm thick) supported on rat skin is shown in Figure 3. The flux of EtAc through these same membranes supported on rat skin is shown in Figure 4. The flux of EtAc is somewhat greater through the more rubbery 18% VAc copolymer relative to the 12% VAc content EVAc membrane.

Table II—Flux of Levonorgestrel, Ethyl Acetate, and Ethanol Through Membrane/Rat Skin Systems[a]

Membrane (% VAC)	Thickness, μm	Steady-State Flux[b]		
		LN	EtAc	EtOH
EVAc (12)	50	1.1	7.7	5.5
	200	0.15	1.5	2.2
EVAc (15)	50	1.0	9.0	7.1
EVAc (18)	50	2.1	9.9	7.9
EVAc (25)	50	1.3	8.1	9.5

[a] Donor phase used as EtAc:EtOH (7:3, v/v) saturated with excess LN. [b] Flux of LN expressed as μg/cm^2·h; flux of EtAc and EtOH expressed as mg/cm^2·h.

Discussion

Levonorgestrel is an extremely potent contraceptive steroid capable of suppressing ovulation at a delivery rate as low as 20 μg/d from implants.[19] The National Institute of Child Health and Human Development has suggested a delivery rate of 35 to 40 μg/d to ensure complete suppression of ovulation. The development of a once-a-day transdermal delivery system for LN requires a permeation enhancer as LN alone is insufficiently permeable to reach the delivery rate of 35 to 40 μg/d. A number of chemicals were tested to increase the skin permeability of LN so that a relatively small patch (5 to 10 cm^2) could be used. Ethyl acetate, either alone or mixed with EtOH, was found to very effectively increase the skin permeability.[13]

The LN delivery rate from a 5-cm^2 patch would need to be ~0.3 μg/cm^2·h. We have used rat skin to evaluate most of the enhancers. Animal skins are generally more permeable than is human skin;[20–22] this is true in the case of LN. We found that using EtOH as an enhancer, the flux of LN through human skin was three to four times less than that through rat skin.[10] Therefore, the target flux for LN in the in vitro experiments using rat skin was higher (1.0 μg/cm^2·h) when considering a 5-cm^2 patch. Even with such a correction factor, rodent skin is a poor model for human skin, making extrapolation to human skin difficult.

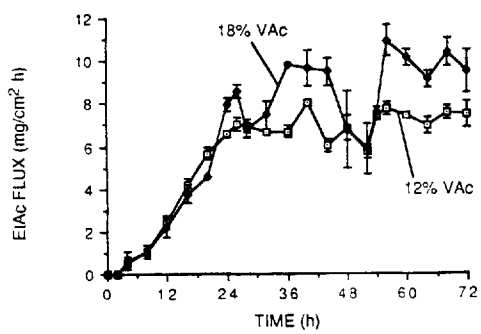

Figure 4—Flux of EtAc through EVAc membranes (18 and 12% VAc content, 50 μm thick) supported on rat skin. The donor phase was EtAc:EtOH (7:3, v/v) gelled with 2 wt % hydroxypropyl cellulose. Error bars are mean standard error.

The use of EtAc in a transdermal formulation requires a reservoir to hold sufficient amounts of EtAc:EtOH to effectively increase the skin permeability towards LN over a period of 24 h. As such, a membrane is required to contain the drug:reservoir suspension and, if needed, to control the delivery of LN or enhancer(s) to the skin. The data presented herein would suggest that EVAc membranes can be used to control the rate of EtAc:EtOH delivery to the skin. By simply selecting the VAc content and thickness, the appropriate LN delivery rate can be obtained.

These experiments were performed with an infinite reservoir of EtAc:EtOH. A transdermal device will contain a finite amount of enhancer solvent. In the design of a transdermal device, the amount of solvent required over the lifetime of the device must be considered. The total amount of EtAc:EtOH delivered over a 24-h period using the standard donor phase (EtAc:EtOH, 7:3) can be estimated from the flux experiments using membranes supported on rat skin. For example, using a 50-μm thick EVAc membrane (18% VAc), ~18 mg/cm^2·h, or a total of 2.1 g of total solvent, would be delivered from a 5-cm^2 device through rat skin. These estimates are for rat skin, which is generally more permeable than is human skin.[20–22] Therefore, the reservoir volume requirements may not be as great as estimated above for rat skin.

Full-thickness excised rat skin was used in this work. It has been reported that full-thickness skin represents an artificially high barrier towards the percutaneous absorption of hydrophobic compounds.[23,24] This has been attributed to the dermal tissues, which, being essentially an aqueous barrier, inhibit the partitioning of hydrophobic substances from the lipophilic stratum corneum. Clearance of hydrophobic drugs in vivo from the viable tissues is believed to be much more efficient than is observed in vitro due to the microvasculature close to the surface of the skin. The long approach to steady state observed in our in vitro experiments is probably due to a similar effect (i.e., without an efficient clearance mechanism, partitioning into the viable tissues is a slow process). Indeed, experiments using transdermal devices delivering LN in vivo[25] indicate that LN is absorbed much more rapidly than would be expected based on the in vitro results.[10,11,13,14]

Safety and skin irritation are of concern with any new potential skin permeation enhancer. Ethyl acetate, as is butyl acetate, is classified as Generally Recognized as Safe (GRAS)[11] by the FDA. It is relatively low in toxicity (LD_{50} for acute oral toxicity is 5.6 g/kg), and it is hydrolyzed in vivo to ethanol and acetic acid with a $t_{1/2}$ of 5 to 10 min.[26] The joint FAO/WHO Expert Committee on Food Additives has given EtAc an unconditional "Acceptable Daily Intake (ADI)" of 0–25 mg/kg.[27] Ethyl acetate (10% in petrolatum base) has been shown to be nonirritating and nonsensitizing in occlusive patch tests on humans over a period of 48 h,[27] and to affect the horny layer to about the same extent as does EtOH with respect to transepidermal water loss.[28]

References and Notes

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