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Report

Transdermal Delivery of Levonorgestrel. V. Preparation of Devices and Evaluation *in Vitro*

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Transdermal devices were prepared and evaluated for their ability to codeliver levonorgestrel and the permeation enhancers ethyl acetate and ethanol *in vitro*. The 24-hr devices were prepared with membranes composed of ethylene vinyl acetate (EVAc) copolymers. The vinyl acetate (VAc) content of the membranes (50 ± 10 or 100 ± 10 μm thick) was varied from 12 to 25% to give a range of permeabilities toward the enhancers. The reservoir used was ethyl acetate/ethanol (7:3, v/v; 0.5 ml) containing excess solid levonorgestrel and gelled with 2% hydroxypropyl cellulose. The higher VAc content membranes (18 and 25%) exhibited relatively high release rates of EtAc and EtOH leading to depletion of ethyl acetate and ethanol from the reservoir by the end of 24 hr. As a result, the transdermal flux of levonorgestrel, evaluated using rat skin, reached a maximum at about 8 hr and thereafter diminished to zero by 24 hr. The less permeable membranes (12 and 15% VAc content) led to a more sustained release of enhancers, but due to lower solvent delivery to the skin, levonorgestrel flux was substantially lower. There was a direct relationship between drug delivery through skin and the amount of solvent delivered until release of the enhancers had diminished. The potential use of ethyl acetate in transdermal drug delivery is also discussed.

KEY WORDS: levonorgestrel; transdermal delivery; permeation enhancers, ethyl acetate; ethanol; ethylene vinyl acetate copolymers.

INTRODUCTION

Levonorgestrel is a very potent contraceptive steroid capable of suppressing ovulation at delivery rates as low as 20 μg/day from implants (1). Thus, its delivery via the transdermal route is suggested. Even at this low dose requirement, levonorgestrel alone is not sufficiently skin permeable to meet the daily target delivery rate. Therefore, we have investigated the use of permeation enhancers (2,3) as well as prodrug modification (4) to increase the skin permeability of levonorgestrel. We found that ethyl acetate (EtAc) either alone or in combination with ethanol (EtOH) is a very effective permeation enhancer for levonorgestrel as well as for a variety of other drugs (3,5).

Preparing a transdermal delivery system for levonorgestrel with EtAc/EtOH as a permeation enhancer cosolvent system 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. A reservoir-type device was therefore chosen to codeliver drug and enhancer(s) to the skin. A variety of membranes was evaluated for the ability to contain the drug/enhancer suspension and, if needed, to control the delivery of drug or enhancer(s) to the skin. It was found that ethylene vinyl acetate copolymer membranes were suitable for codelivery of drug and enhancers at appropriate rates (6).

These membranes were incorporated into reservoir-type devices for testing both *in vitro* and *in vivo*. This paper describes the preparation of the devices and the release of levonorgestrel, EtAc, and EtOH under sink conditions and through excised rat skin.

MATERIALS AND METHODS

Levonorgestrel (micronized) was a gift of the World Health Organization. EtAc (reagent grade) and hydroxypropyl cellulose were purchased from Aldrich Chemical Co. (Milwaukee, Wis.). Ethanol (200 proof, U.S.P./N.F. grade) was purchased from USI Chemicals (Tuscola, Ill). The chemicals were used as received. Ethylene vinyl acetate (EVAc) copolymers were obtained from DuPont (Wilmington, Del.). Backing (Scotch 1220) and release liner (Scotch 1022) were obtained from 3M Company (St. Paul, Minn.). The medical-grade adhesive BIO-PSA X7-2920 was a gift of Dow Corning (Midland, Mich.). The rats (male, Wistar strain, 180 to 220 g) were obtained from Simensen Labs (Gilroy, Calif.).

Preparation of Polymeric Membranes. Homogeneous films were prepared by solvent casting or heat pressing. Previous work has indicated that there is no significant difference in the observed permeability of drug or solvents through membranes prepared by either technique. EVAc membranes (25% VAc content) were prepared by dissolving

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the polymer pellets in trichloroethylene at a 10 wt% concentration. The solution was cast onto glass plates, allowed to cure a minimum of 12 hr at room temperature, then removed from the plate by flotation on water. Curing under vacuum or heat was not required to cure the membranes. EVAc membranes (12, 15, and 18% VAc content) were prepared by heating the polymer pellets near the Vicat softening point (7) of the polymer. Pellets were placed between Teflon-faced aluminum sheets, heated, then pressed at 30,000 psi. After cooling, the finished films were graded for uniformity, and the thickness was measured at three points with a Mercer caliper snap gauge, which is accurate down to 2 μm. Membranes were prepared at a thickness of either 50 ± 10 or 100 ± 10 μm.

Preparation of Transdermal Devices. A device area of 4.91 cm^2 was selected as the active membrane area of the test devices. Heat-sealed devices were prepared with a spacer to allow for modification of the reservoir volume. All heat-sealing operations were performed at a temperature of 120°C. A high-density polyethylene ring was first heat-sealed to the backing material (Scotch 1220, a clear polyester film coated with a heat-sealable polyethylene layer). Membrane/adhesive laminates were prepared by casting a 100-μm-thick film of BIO-PSA X7-2920 medical-grade adhesive onto Scotch 1022 release liner (a fluoropolymer-treated polyester film). BIO-PSA X7 2920 is supplied as 45% solids dissolved in trichlorotrifluoroethane (Freon PCA). After allowing the adhesive to cure (about 1 min, which is adequate to remove the Freon), the appropriate EVAc membrane was applied to the adhesive/release liner laminate. The membrane/adhesive laminate was then heat-sealed to the polyethylene spacer to form an empty device. A release slip composed of Scotch 1022 was used to leave a small opening into the reservoir of the empty devices. After the devices were sealed completely, the slip was removed to form a port and a gelled levonorgestrel suspension (500 μl) was injected into the reservoir. The drug suspension was prepared by mixing levonorgestrel into EtAc/EtOH (7:3 v/v) at 1.5 wt%. After stirring (200 rpm) for 1 hr, 2 wt% of hydroxypropyl cellulose (M_w = 1,000,000) was slowly sifted into the vortex of the stirring suspension. The gelled suspensions were then stirred for an additional 1 hr at high speed (1,000 rpm), sonicated for several minutes to remove entrapped air, and allowed to stand overnight. After injecting the gelled levonorgestrel suspension into the devices, the port used was heat-sealed. The devices were then punched free to obtain a finished patch.

Release of Levonorgestrel, EtAc, and EtOH from Devices. The release of levonorgestrel, EtAc, and EtOH from the transdermal devices was performed using a release flask apparatus, consisting of a constant temperature bath into which 1.0-liter flasks were placed. The flasks contained 1.0-liter of receiving phase (normal saline with 0.05 wt% sodium azide added as an antibacterial agent). Test devices were suspended in the flasks, which were sealed to prevent loss of the volatile solvents. The temperature was maintained at 32°C and the receiving phase was stirred throughout the experiment. Samples were removed from the flasks at intervals with replacement volumes and tested for released levonorgestrel, EtAc, and EtOH content by high-pressure liquid chromatography (see below).

Permeability Studies Using Rat Skin in Vitro. A system employing nine glass Franz diffusion cells was used to test the release of levonorgestrel, EtAc, and EtOH from the transdermal devices through excised rat skin. The Franz cells were modified with inlet and outlet receiver phase ports to allow continuous flow through the cells. The rats 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 skins were washed with physiological saline and used in the permeability experiment within 1 hr. The devices were applied to the skins following removal of the release liner. The skins, with devices attached, were then mounted and clamped between the cell body and the cell cap; the epidermal side was faced upward (donor side). The receptor phase, in contact with the underside of the skin, was isotonic saline (0.1 wt% sodium azide added) which was maintained at 37°C. The cells were maintained at 37°C by thermostatically controlled water which was circulated through a jacket surrounding the cell body. 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 below 10% of saturation. Uniform mixing of the drug in the receiver phase was achieved by a small magnetic stirring bar driven by an external 600 rpm motor. Fractions were collected every 2 hr in test tubes. Flux was calculated by measuring the total amount of drug or enhancer collected in the 2-hr period and dividing this by 2 to obtain an hourly rate.

Chromatographic Analysis. Levonorgestrel in the receptor phase from release and skin permeation experiments 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 analytical column used for levonorgestrel was a 4.6-mm × 25-cm, 10-μm, Whatman ODS-3 Partisil C-18. The mobile phase used was acetonitrile/H_2O (50:50, v/v) at a flow rate of 2.0 ml/min with UV absorbance monitoring at 243 nm. The retention time of levonorgestrel was 6.0 min. EtAc and EtOH were measured with a Waters Fast Fruit Juice 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. 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. For *in vitro* work using Franz cells, standards of EtAc and EtOH were used to correct for the loss of these two volatile solvents from the effluent collected in the test tubes on the fraction collector prior to analysis by HPLC.

RESULTS

Preparation of Devices

The transdermal devices used in this study were prepared by heat-sealing the various polymeric components. The overall procedure used to prepare the devices is shown in Fig. 1. The membranes used in the devices were EVAc

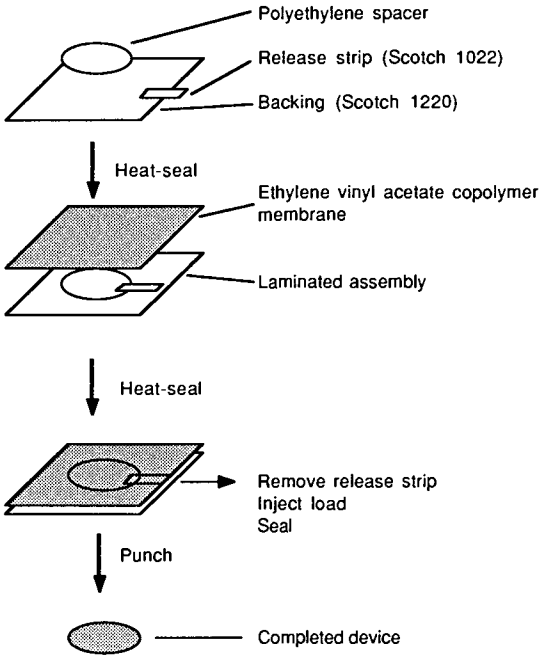

Fig. 1. Schematic representation of transdermal patch preparation.

films prepared at a thickness of 50 ± 10 or 100 ± 10 μm. The VAc content was varied from 12 to 25%, giving a range of permeabilities. It has been shown that for most low molecular weight chemicals, the permeability of EVAc membranes increases with increasing VAc content (8,9).

Release of Levonorgestrel, EtAc, and EtOH from Devices

Release of levonorgestrel, EtAc, and EtOH from devices was measured over a 24-hr period. The devices were suspended in 1.0-liter flasks; samples were periodically withdrawn and analyzed for released drug and enhancers. Cumulative release profiles of levonorgestrel from devices using EVAc membranes (12, 15, 18, and 25% VAc content; 100 ± 10 μm thick) are shown in Fig. 2. Release of levonorgestrel varied depending on the VAc content of the EVAc membrane: the 25% VAc content membrane released slightly more drug over 24 hr relative to the other three membranes tested. The delivery of EtAc from these same devices

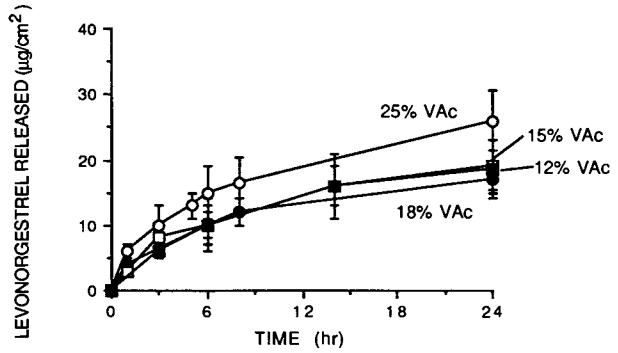

Fig. 2. Cumulative release of levonorgestrel over 24 hr from transdermal devices using 12, 15, 18, or 25% VAc content EVAc membranes (100 ± 10 μm). Error bars represent the high and the low values obtained (N = 3).

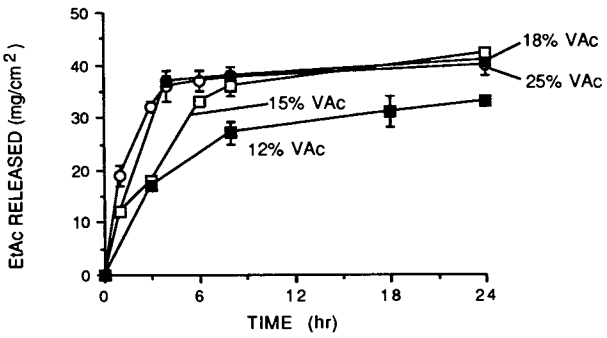

Fig. 3. Cumulative release of EtAc over 24 hr from transdermal devices using 12, 15, 18, or 25% VAc content EVAc membranes (100 ± 10 μm). Error bars represent the high and the low values obtained (N = 3).

is shown in Fig. 3. Most EtAc was delivered within 4 to 8 hr. The 12% VAc content membrane sustained the release of EtAc to a greater extent than the other membranes. The total amount of EtAc released over the 24-hr test period ranged from 160 to 200 mg. Thus, approximately two-thirds of the EtAc was delivered based on the total amount of EtAc in the device at the start of the experiment (290 mg). Delivery of EtOH from these devices is shown in Fig. 4. Release of EtOH was about the same for all the devices except the device prepared with a 12% VAc content membrane, which gave close to constant release over 24 hr. Total EtOH delivered was about 125 mg (with the exception of the 12% VAc membrane), which represents all the EtOH in the device at the start of the experiment.

Permeability Studies Using Rat Skins *in Vitro*

The devices (4.91 cm²) were tested for their ability to deliver levonorgestrel through excised rat skin *in vitro*. Freshly prepared full-thickness rat skins were used in modified Franz diffusion cells. The release liner was removed and the patches placed on the rat skin; the adhesive BIO-PSA X7-2920 held the devices firmly in place throughout the course of the experiment. Levonorgestrel, EtAc, and EtOH permeating through the skins were measured by HPLC. In some cases, the patch was removed after 24 hr, and a new patch placed on the same skin for an additional 24 hr. If not replaced, the patches were left on the skin until the conclu-

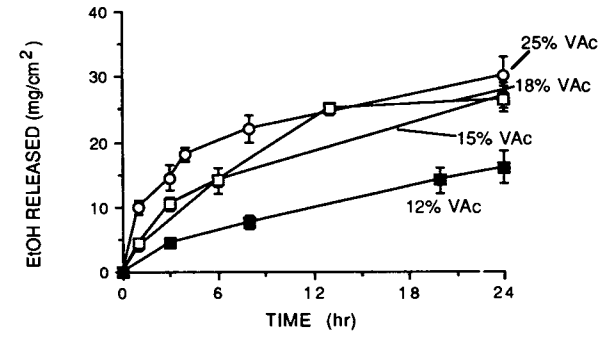

Fig. 4. Cumulative release of EtOH over 24 hr from transdermal devices using 12, 15, 18, or 25% VAc content EVAc membranes (100 ± 10 μm). Error bars represent the high and the low values obtained (N = 3).

sion of the experiment (46 to 48 hr). Membranes tested in the devices were selected based on results of previous *in vitro* experiments (6).

Delivery of levonorgestrel, EtAc, and EtOH from transdermal devices (25% VAc content EVAc membranes, 100 ± 10 μm thick) through rat skin is shown in Fig. 5. The EVAc membrane used in these devices was 25% VAc at a thickness of 100 ± 10 μm. Flux of levonorgestrel reached a maximum at about 16 hr and slowly diminished until a new patch was placed on the skin at 24 hr. The flux of levonorgestrel increased after the new patch was placed on the skin, although total delivery of levonorgestrel over the second 24-hr period was less than that of the first. The reasons for this are unclear: it appears that the skin exhibited increased barrier properties toward all the species released from the device. The lower flux of levonorgestrel from the second patch appears to be related to lower enhancer delivery through the rat skin. Also shown in Fig. 5 is the solvent flux from these devices through rat skin. Maximum flux of both EtAc and EtOH was reached at about 8 hr; thereafter the flux dropped off to essentially zero by 24 hr.

Devices using progressively less permeable membranes (18, 15, and 12% VAc content EVAc membranes) were tested in the same manner. Results using membranes of 18% VAc content are shown in Fig. 6. In this case, the device was left on the skin for 48 hr. Delivery of levonorgestrel was similar to that found from devices with 25% VAc content EVAc membranes: maximum flux was about 0.6 μg/(cm^2 hr). Solvent flux was also similar over the experimental time period.

Results using 15% VAc content EVAc membranes (100 ± 10 μm thick) in the transdermal devices are shown in Fig. 7. In this case, delivery of levonorgestrel was much more constant relative to that observed from the 18 and 25% VAc content EVAc membranes. However, the maximum delivery rate of levonorgestrel was much less in this case: 0.13 μg/(cm^2 hr). The delivery of EtAc and EtOH through the skin was correspondingly less than that observed in devices with more permeable membranes. Maximum enhancer flux of the two solvents was reached at about the same time (8 hr)

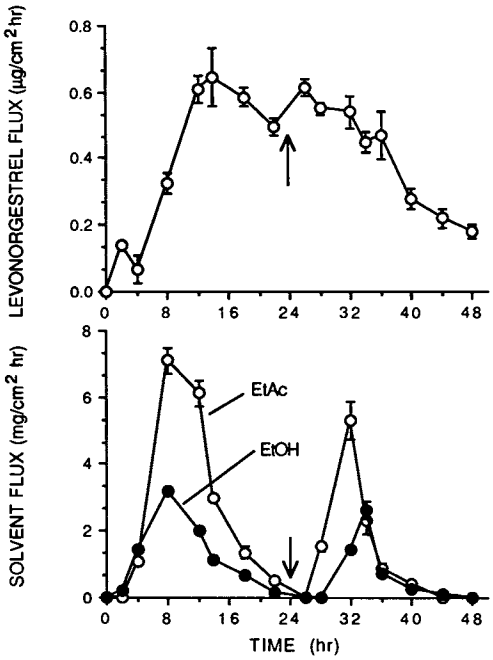

Fig. 5. Release of levonorgestrel, EtAc, and EtOH from transdermal devices (EVAc membranes, 25% VAc content, 100 ± 10 μm thick). The arrow indicates were the device was removed and a new device placed on the rat skin. Error bars represent SE (N = 4).

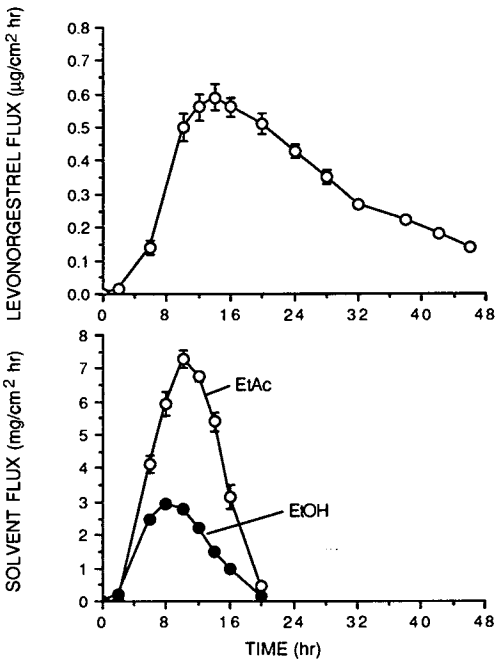

Fig. 6. Release of levonorgestrel, EtAc, and EtOH from transdermal devices (EVAc membranes, 18% VAc content, 100 ± 10 μm thick). Error bars represent SE (N = 4).

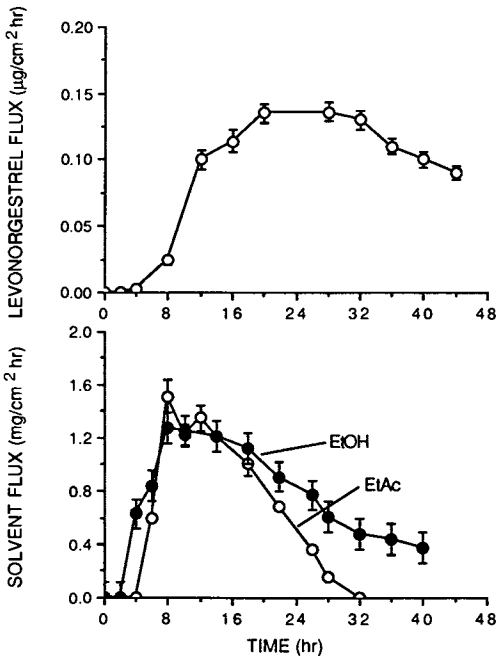

Fig. 7. Release of levonorgestrel, EtAc, and EtOH from transdermal devices (EVAc membranes, 15% VAc content, 100 ± 10 μm thick). Error bars represent SE (N = 4).

but the amount of EtAc delivered was less relative to the devices prepared with more permeable membranes. As a result, the enhancement effect for levonorgestrel was reduced.

The effect of decreased membrane permeability was also apparent in the lowest VAc content EVAc membrane tested (12% VAc) (Fig. 8). Delivery of levonorgestrel from these devices through rat skin was relatively low: 0.08 μg/(cm^2 hr). These membranes restricted flux of EtAc and EtOH, leading to a more sustained solvent delivery relative to that of the more permeable membranes.

The 18 and 25% VAc content EVAc membranes did not appear to impede the delivery of EtAc and EtOH to rat skin. This is consistent with experiments conducted with the same membranes supported on rat skin (6). As such, delivery of levonorgestrel through rat skin was relatively high. As the release of EtAc and EtOH to the skin diminished after 8 hr, delivery of levonorgestrel dropped correspondingly. Devices prepared with more permeable membranes were apparently depleted of EtAc and EtOH before 24 hr. Devices prepared with less permeable membranes delivered less enhancer, leading to a more sustained but lower rate of levonorgestrel delivery. The same trend was observed for the EVAc membranes tested at a thickness of 50 ± 10 μm.

The relationship between solvent delivery and drug delivery from all four formulations is shown in Fig. 9. From this relationship, it appears that flux enhancement is directly related to the amount of solvent delivered to the skin. This relationship holds until solvent release diminishes, at which time drug release continues, which is probably due to drug release from the stratum corneum (reservoir effect). There was also a linear relationship between levonorgestrel delivery and EtAc/EtOH delivery through skin when comparing peak drug and solvent release. The 25% VAc and 18% VAc

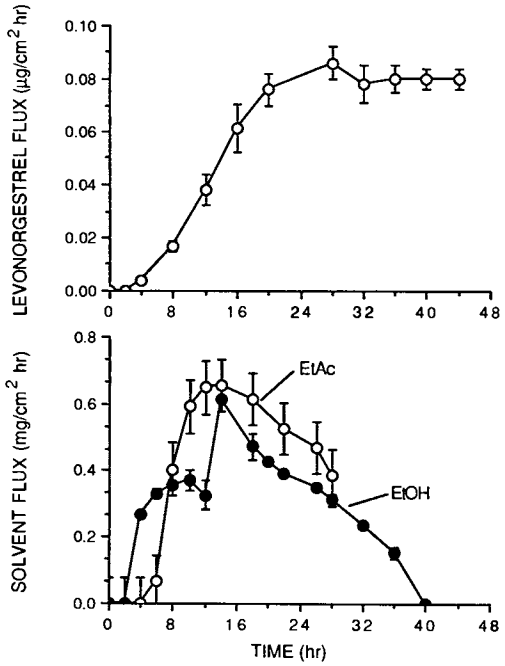

Fig. 8. Release of levonorgestrel, EtAc, and EtOH from transdermal devices (EVAc membranes, 12% VAc content, 100 ± 10 μm thick). Error bars represent SE (N = 4).

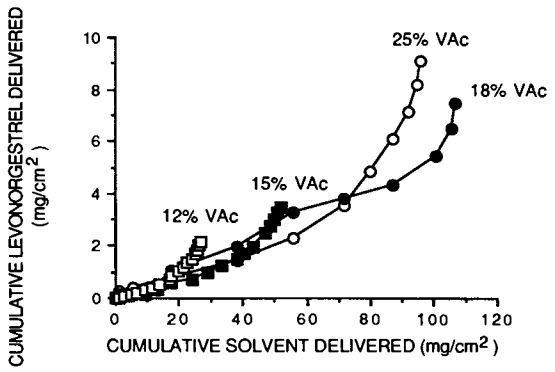

Fig. 9. Relationship between cumulative solvent delivered through rat skin and cumulative levonorgestrel delivered from devices (12, 15, 18, and 25% VAc content EVAc membranes, 100 ± 10 μm thick)

content membrane devices delivered equal amounts of drug and enhancer. The 15% VAc content membrane delivered four times less solvent: drug flux was about four times less. The 12% VAc content membrane restricted solvent delivery about eightfold relative to the 18 and 25% VAc content membrane systems. As a result, drug delivery was about eightfold less. The relationship between drug delivery and solvent and the fact the lag times were about the same for all the formulations indicate that the solvents EtAc and EtOH are primarily increasing drug solubility (partitioning) in the skin (10).

DISCUSSION

The development of a small (5- to 10-cm^2), once-a-day transdermal delivery system for levonorgestrel requires a very effective penetration enhancer. EtAc, either alone or in combination with EtOH, meets this requirement. The target delivery rate of levonorgestrel for contraception, as established by the National Institute of Child Health and Human Development, is 35 to 40 μg/day. To meet this target for a 5-cm^2 patch, delivery of levonorgestrel would need to be about 0.3 μg/(cm^2 hr). Rat skin has been used to evaluate our enhancers and devices. As rodent skins are generally more permeable than human skin (11–13), the target flux for rat skin must be adjusted accordingly. With EtOH as an enhancer, the flux of levonorgestrel through human skin was about three to four times less than that through rat skin (2). Using EtAc as an enhancer, the flux through human skin was four times less (14). Therefore, the target flux for levonorgestrel through rat skin is higher [1.0 μg/(cm^2 hr)] when considering a 5-cm^2 patch.

The results from the present study indicate that a device can be prepared using EtAc/EtOH as a skin permeation enhancer cosolvent system. The device components are stable toward EtAc/EtOH, although long-term stability studies will need to be performed to assess shelf-life of an EtAc-based transdermal delivery system. The adhesive BIO-PSA X7-2920 maintains its adhesive properties in the presence of EtAc/EtOH with no apparent change over 24 hr.

The maximum flux of levonorgestrel from devices with the most permeable membrane (EVAc, 25% VAc content) through rat skin was close to the target flux. In previous work, the steady-state flux of levonorgestrel through rat skin using a EtAc/EtOH (7:3) enhancer solvent system as a donor

phase was found to be about 0.9 μg/(cm^2 hr) (3). In contrast, the flux of levonorgestrel through rat skin from a donor phase of 100% EtOH was only about 0.06 μg/(cm^2 hr) (2). The highest flux obtained from the devices was about 0.6 μg/(cm^2 hr). It was probable that the devices (18 and 25% VAc content membranes), with their limited reservoir volume, were depleted of EtAc/EtOH well before 24 hr had elapsed. Increasing the reservoir volume would allow more enhancer solvent to be delivered to the skin over the 24-hr period, leading to a higher and more sustained delivery of levonorgestrel. A larger active surface area could also be used to meet the target delivery flux. Another possibility is to use a patch in which the reservoir area is larger than the active drug delivery area. Such a patch would be acceptably thin but still capable of delivering adequate amounts of solvent over the life time of the device.

These experiments, as were previous experiments with levonorgestrel (2–6), were performed using full-thickness rat skin. Full-thickness skin *in vitro* represents an artificially high barrier to absorption of lipophilic compounds relative to the same skin *in vivo* (15–17). This artificial barrier *in vitro* is thought to arise from the lack of blood capillaries that are present *in vivo* to help clear low-water-soluble drugs. A significant unstirred water layer is present in full-thickness skin *in vitro* as well. To help improve the *in vitro/in vivo* correlation, special receptor phases [40% PEG in saline (18), 3% bovine serum albumin (19)] and relatively thin skin (200 μm thick) (16) have been used. The data collected using full-thickness rat skin indicate that it is possible to formulate a transdermal patch using EtAc/EtOH as enhancers and that, by selecting membranes of various permeabilities, the delivery of levonorgestrel through skin can be controlled. It is not indicative of results to be expected in humans; however, the same approach can be used to find the proper device components for a delivery system for human use.

EtAc is susceptible to both chemical and enzymatic hydrolysis to EtOH and acetic acid. Some of the EtOH detected in the receptor phase was probably due to hydrolyzed EtAc. In experiments using pure EtAc as an enhancer, we observed EtOH in the receptor phase, indicating that 10 to 20% of EtAc was hydrolyzed to EtOH (and acetic acid) (14).

The lag times for the various species (drug and enhancer) must be considered in determining the feasibility of using a once-a-day transdermal delivery system for levonorgestrel. The lag time of levonorgestrel through rat *in vitro* using EtAc/EtOH (7:3) as a donor phase is about 18 hr (3). In human skin, the lag time is closer to 24 hr. From transdermal devices, the lag times of levonorgestrel through human skin *in vitro* has been measured from 24 to 34 hr, depending on the enhancer composition (20). The lag time was the same using saline or 40% PEG/saline as a receptor phase (14). The relatively long lag times may be due to binding in the epidermis, which is known to occur with other steroids (21). The lag time of EtAc and EtOH through rat skin or human cadaver skin is 10 to 12 hr under *in vitro* conditions (14). The lag times of levonorgestrel, EtAc, and EtOH *in vivo* are probably shorter. For example, in rabbits the lag time of levonorgestrel delivered from a device containing EtAc and EtOH was found to be 2 to 4 hr, depending on the membrane employed in the device (22). More experiments will need to be performed *in vivo* to determine if the lag times are short enough to make a once-a-day transdermal delivery system feasible for levonorgestrel using EtAc/EtOH as enhancers.

Safety and skin irritation must be considered with all proposed permeation enhancers. EtAc is Generally Recognized as Safe (GRAS) by the Food and Drug Administration. It is relatively low in toxicity: LD_{50} is 5.6 g/kg for acute oral toxicity and 3 to 5 g/kg for subcutaneous toxicity (23). EtAc is hydrolyzed *in vivo* to EtOH and acetic acid with a $t_{1/2}$ of 5 to 10 min (24). EtAc (10% in petrolatum base) was found to be nonirritating and nonsensitizing in occlusive patch tests on humans over 48 hr (23). EtAc affects the horny layer to about the same extent as does EtOH with respect to transepidermal water loss (25). EtAc has also been used to study sebum production and delivery in man (26). These workers found that EtAc was a very good delipidizing agent, which may help to explain the enhancement effect observed when EtAc is applied to the skin. The devices tested herein have been evaluated for skin irritation on rabbits. The patches produced mild erythema and very mild edema following a 24-hr exposure period (27). These changes were found to be reversible. Current work is focused on studying the mechanism of action of EtAc relative to that of other enhancers such as EtOH, laurocapram (Azone) (28,29), and dimethyl sulfoxide (30).

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