ENHANCEMENT OF TRANSDERMAL DELIVERY BY SUPERFLUOUS THERMODYNAMIC POTENTIAL. II. 1) IN VITRO-IN VIVO CORRELATION OF PERCUTANEOUS NIFEDIPINE TRANSPORT*

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Nifedipine was selected as a representative compound to investigate a method for improving transdermal bioavailability. The general strategy explored to improve the percutaneous transport of nifedipine was the manipulation of thermodynamics of the drug substance by the use of volatile/nonvolatile systems as vehicles. To investigate the potential of the strategy, diffusion studies were conducted using an ethylene-vinyl acetate copolymer (EVA) membrane and full-thickness excised abdominal skin of rats. Little penetration through EVA membrane or rat skin was found either from the volatile solvent ethanol (EtOH) or from the nonvolatile solvent diethyl sebacate (DES). When the vehicle was changed to a mixed solvent containing both EtOH and DES in a volume ratio of 75:25, penetration through EVA membrane or rat skin was increased up to 3 to 4 times, compared with those values for DES. The increase in the penetration was accounted for by the increase in the thermodynamic activity of the drug in the nonvolatile vehicle caused by the evaporation of the volatile component. The bioavailability of percutaneous nifedipine in rats was determined from the drug solutions containing different proportions of EtOH and DES. Once again, the highest bioavailability was achieved from the mixed solvent containing EtOH and DES in a volume ratio of 75:25. The area under the plasma nifedipine concentration-time curve for the mixed solvent was higher by about 4 times than that for DES.

Keywords — in vitro-in vivo correlation; transdermal delivery; nifedipine; penetration enhancement; thermodynamic potential; supersaturation; volatile component; diffusion model; bioavailability; rat

Percutaneous administration of nifedipine could be an alternative in the management of hypertension. 2) However, the penetration of nifedipine through a lipoidal membrane has been shown to be poor, 1) and extensive surface area may be needed for therapeutic effect. Therefore attempts to enhance penetration may be worthwhile for good patient acceptance and compliance.

Using in vitro techniques the enhanced penetration of nifedipine through an artificial membrane has been shown with vehicle mixtures of volatile/nonvolatile solvent systems. 1) The final assessment of bioavailability from these systems can only be obtained from in vivo studies in man. However, for ethical reasons, in vitro methods and an animal model have been used during the product development.

The purpose of this investigation was to compare the bioavailability of percutaneous nifedipine from volatile/nonvolatile mixed solvent systems. The only difference between the tested vehicles was the proportions of ethanol (EtOH) and diethyl sebacate (DES) used. These were characterized by different in vitro penetrations of nifedipine, and in vitro-in vivo correlations are discussed.

MATERIALS AND METHODS

All procedures were carried out under subdued lighting conditions due to the high sensitivity of nifedipine to light.

Materials — Nifedipine JP XI grade was used. An internal standard (I.S.), diethyl 2,6-dimethyl-4-(2-nitrophenyl)-3,5-pyridinedicarboxylate, was synthesized according to the procedure reported previously. 3) All other chemicals were commercial products of reagent grade. Ethylene/vinyl acetate copolymer (EVA) membrane (composition: ethylene-vinyl acetate, 90/10, thickness: 40 μm) obtained from Tamapoly Co., Ltd., Tokyo, Japan was used. Male Wistar rats
(186–225 g) were used.

**Vehicle Preparations** — Test solutions were prepared to contain 10 mg/ml of nifedipine in pure EtOH, DES and in mixed solvents consisting of EtOH–DES in volume ratios of 25:75, 50:50 and 75:25. EtOH is miscible with DES in all proportions. Nifedipine is sufficiently soluble in these solvents or mixed solvents.

**In Vitro Diffusion Cell Studies** — A model system for drug transport across the lipidoidal barrier has been studied by a diffusion cell technique employing an EVA membrane and full-thickness excised abdominal skin from rats. The excised skin was obtained from the abdominal surface of a male Wistar rat. After the removal of the abdominal hair with electric clippers and an electric shaver, the skin was excised from the sacrificed animal and the adipose tissue was removed. The diffusion cell (Kersco Engineering Consultants, Palo Alto, California, U.S.A., Fig. 1) was the same as that used by Sloan et al. 4) as described previously. 1) The opening in the lid left exposed an 8.0 cm² area on the barrier membranes through which penetration was measured. The receptor was filled with 45 ml of saline solution incorporated with 0.01% (w/v) thimerosal (preservative). Test formulations (1 ml) were applied on the membrane surface and the volatile solvent allowed to escape. In some experiments silicone grease was smeared around the central well and the top of each cell was sealed with a glass cover slip to prevent evaporation of the solvent. Each cell was placed on a magnetic stirrer

in a thermostated chamber maintained at 37 °C. Samples of 100 μl were removed for analysis from the receptor phase via the side arm at 1, 2, 4, 6, and 8 h after the application.

**Evaporation Studies** — One ml of the nifedipine solution (10, 23 or 50 mg/ml) in a mixed solvent of EtOH and DES in a volume ratio of 75:25 was pipetted to a 5-ml glass tube, 7.5 cm × 1.0 cm i.d., and the volatile component was evaporated under a gentle stream of nitrogen at 37 °C. At the appropriate time after the beginning of the evaporation, the supernatant fluid was filtered, diluted with methanol and its concentration was determined by means of a spectrophotometer at 350 nm.

**In Vivo Animal Studies** — An abdominal dosing site of a male Wistar rat was prepared as described previously. 2) Urethan was administered subcutaneously as a 200 mg/ml solution at a dose of 1.0 g/kg to induce continuous anesthesia during the experiment and the animal was restrained in the supine position on a 37 °C surface. A silicone chamber was applied to the surface of the abdomen using a cyanoacrylate adhesive (Aron Alpha, Toa Gosei Chemical Co., Ltd., Tokyo, Japan). Doses (0.5 ml) were placed in the chamber and the volatile solvent allowed to escape (Fig. 2). The surface area available for absorption was 2.0 cm². At appropriate intervals, 0.3 ml of blood was collected from the jugular vein. Blood samples were centrifuged and 0.1 ml plasma samples were removed for analysis.

**Nifedipine Assay** — Measurement of nifedipine concentrations in the receptor fluid and plasma was done by gas-liquid chromatography using an electron capture detector. 3)
RESULTS AND DISCUSSION

*In Vitro Diffusion Cell Studies*

Because human skin specimens from surgical procedures or from cadavers are difficult to obtain and vary in permeability, we used other materials to simulate the transdermal absorption of nifedipine. Many workers have used diffusion methods with artificial membranes such as silicone rubber, ethylene-vinyl acetate copolymers, polyurethanes and other dense polymers. The skin barrier is generally considered to be lipoid in nature. Thus, an EVA membrane was chosen for its ease of preparation and reproducibility.

Figure 3 shows the time course of nifedipine penetration through an artificial EVA membrane from test solutions at 37 °C. Little penetration was achieved from pure DES. The penetration of nifedipine increased with increasing volume ratio of EtOH to DES in the donor solution. When the nifedipine solution in pure EtOH was applied and all the solvent was allowed to evaporate, penetration was very low.

Certain solvents have been shown to affect the barrier function of the EVA membrane so as to promote penetration of the drug. However, it is clear that the enhancement of penetration observed was due to the increase in the solute concentration caused by the evaporation of the volatile component, since the penetration was very low from all the occluded systems where evaporation was minimized (Fig. 4).

The solubility of nifedipine in mixed solvents containing different proportions of EtOH and DES was determined at 37 °C according to the previously presented procedure (Fig. 5). The ratio of the drug concentration (C) to the solubility (S) in the vehicle gives an approximation of the thermodynamic activity. A saturated solution was represented by a ratio of unity. The C/S ratios of nifedipine in test solutions are summarized in Table I. The C/S ratio before
evaporation was calculated by dividing the initial nifedipine concentration (10 mg/ml) by each saturation solubility depicted in Fig. 5. The C/S ratio after evaporation was evaluated by dividing the nifedipine concentration in the remaining DES by its saturation solubility, assuming complete evaporation of EtOH. The average penetration of nifedipine from test solutions after 8 h with or without occlusion, depicted in Fig. 4, follows a similar pattern and relates closely to the C/S ratios given in Table I.

On application to the membrane surface, 1 ml of EtOH evaporates within 10 min at 37 °C. Since the solubility of nifedipine in DES is 22.9 mg/ml (Fig. 5), a supersaturated solution should develop when 75% of the vehicle has evaporated from the solution in which the initial nifedipine concentration was 10 mg/ml. When the nifedipine solution in a mixed solvent of EtOH and DES in a volume ratio of 75:25 was applied to the membrane and allowed to evaporate at 37 °C, no precipitation was observed with the naked eye during the entire time course of the experiments.

Figure 6 depicts the effect of the initial nifedipine concentration on the drug precipitation in the mixed solvent of EtOH and DES in a volume ratio of 75:25 under a gentle stream of nitrogen at 37 °C. In a system where supersaturation is achieved within a metastable state, precipitation is improbable. In contrast, when supersaturation exceeds a certain limit the precipitation

**TABLE I. The Ratio of Nifedipine Concentration (C) to Its Solubility (S) in Test Solutions before and after Evaporation**

<table>
<thead>
<tr>
<th>Volume ratio (EtOH-DES)</th>
<th>Before evaporation</th>
<th>C/S</th>
<th>After evaporation</th>
</tr>
</thead>
<tbody>
<tr>
<td>0:100</td>
<td>0.44</td>
<td></td>
<td>0.44</td>
</tr>
<tr>
<td>25:75</td>
<td>0.17</td>
<td></td>
<td>0.58</td>
</tr>
<tr>
<td>50:50</td>
<td>0.16</td>
<td></td>
<td>0.87</td>
</tr>
<tr>
<td>75:25</td>
<td>0.19</td>
<td></td>
<td>1.75</td>
</tr>
<tr>
<td>100:0</td>
<td>0.32</td>
<td></td>
<td>—</td>
</tr>
</tbody>
</table>

**FIG. 6. Effect of the Initial Nifedipine Concentration on the Drug Precipitation in a Mixed Solvent of EtOH and DES in a Volume Ratio of 75:25 at 37 °C**

The initial nifedipine concentrations are 10 (●), 23 (★) and 50 (▲) mg/ml. The dotted line shows the saturation solubility of nifedipine in DES.

**FIG. 7. Receptor Phase Concentration - Time Curves of Nifedipine Penetrated through Rat Skin from Test Solutions at 37 °C**

Key: ▼, DES; ■, EtOH-DES (25:75); ▲, EtOH-DES (50:50); ●, EtOH-DES (75:25); ♦, EtOH. Each point represents the average of 3 replicates; bars represent the standard deviations.
occurs very rapidly.

From these observations, we conclude that the superfluous thermodynamic potential of the penetrating agent in the metastable supersaturated solution may result in an increased rate of transport across the lipoidal barrier. A detailed discussion on thermodynamics of supersaturation has been given in the preceding paper.\textsuperscript{11}

Excised skin from rats has been also used in diffusion cells. Figure 7 shows the time course of nifedipine penetration through rat skin from test solutions at 37 °C. Pure EtOH, DES and a mixture of the two solvents in an EtOH–DES volume ratio of 25:75 produced poor penetration of the drug. Once again, the highest penetration was achieved by the mixed solvent of EtOH and DES in a volume ratio of 75:25.

Figure 8 shows the average penetration of nifedipine through rat skin from test solutions after 8 h. A good correlation was found between the EVA data (Fig. 4) and excised skin data (Fig. 8) with the correlation coefficient of linear regression analysis calculated to be 0.936.

The permeability coefficient of nifedipine for each run of the nonvolatile solvent was calculated from the steady-state portion of the receptor compartment concentration versus time profile using:

$$P = \frac{(dQ/dt)}{A\Delta C} = \frac{V(dC/dt)}{A\Delta C} \quad (1)$$

where $P$ is the permeability coefficient, $V$ is the volume of the receptor compartment, $dC/dt$ is the steady-state slope of the concentration-time profile, $A$ is the diffusional area and $\Delta C$ is the concentration difference across the membrane, which was taken to be equal to the initial donor concentration since the buildup of material in the receiver was never allowed to become a significant fraction of that in the donor phase. The permeability coefficients for EVA membrane and excised skin were calculated as $17.6 \times 10^{-9}$ $\pm 4.8 \times 10^{-9}$ cm/s and $5.8 \times 10^{-9} \pm 1.1 \times 10^{-9}$ cm/s (mean ± S.D.), respectively.

**In Vivo Animal Studies**

The plasma nifedipine concentration–time curves obtained following percutaneous administration of test solutions to rats are shown in Fig. 9. Plasma nifedipine concentration at 2 h after application of the drug solution in DES reached a plateau level (about 30 ng/ml). As depicted in Fig. 9, transdermal absorption was enhanced when the formulation was changed to contain both EtOH and DES. The highest plasma nifedipine concentration was achieved by the mixed solvent of EtOH and DES in a volume ratio of 75:25. When the nifedipine solution in pure EtOH was applied and all the solvent was allowed to evaporate, the plasma nifedipine concentrations were very low.

Figure 10 shows the average bioavailability of

![Graph showing average penetration of nifedipine through rat skin from test solutions after 8 h at 37 °C.](image)

**FIG. 8.** Average Penetration of Nifedipine through Rat Skin from Test Solutions after 8 h at 37 °C

Each point represents the average of 3 replicates; bars represent the standard deviations.

![Graph showing plasma nifedipine concentration–time curves following percutaneous administration of test solutions.](image)

**FIG. 9.** Plasma Nifedipine Concentration–Time Curves Following Percutaneous Administration of Test Solutions

Key: ▼, DES; ■, EtOH–DES (25:75); ▲, EtOH–DES (50:50); ○, EtOH–DES (75:25); ◆, EtOH. Each point represents the average of 3 rats; bars represent the standard deviations.
nifedipine, that is the area under plasma concentration–time curve (AUC) of the drug from zero to 8 h, from test solutions. The data obtained from the in vitro diffusion and bioavailability studies in rats were subjected to linear least-squares regression analysis. The correlation coefficients between in vitro penetration through an EVA membrane or excised skin and AUC were calculated as 0.893 and 0.868, respectively.

The applied volume per unit area for in vivo studies differed from the in vitro studies. On application into the silicone chamber adapted on the rat abdomen, 0.5 ml of EtOH evaporates within 40 min. These physical factors, such as applied volume and environmental temperature, might result in weak correlations between in vitro data and in vivo data.

The permeability coefficient for nifedipine in the skin barrier of a living animal was estimated in the following manner. The time course of the intact drug amounts absorbed into the systemic circulation from a nifedipine solution in DES was simulated (Fig. 11) by a numerical deconvolution method as described previously. Using Eq. 1, the in vivo permeability was calculated to be $25.4 \times 10^{-9} \pm 0.6 \times 10^{-9}$ cm/s (mean ± S.D.). This value is about 4 times that of excised skin. A possible explanation is that a true in vivo diffusion area may be larger than the area of skin to which drug is applied, since diffusion in the stratum corneum can be multidirectional in a real situation. In addition, an interpretation of an in vitro diffusion experiment may be in error when in situ hydration of the stratum corneum membrane by water diffusing from receptor solution closes down the shunt routes. Drugs which may well pass down the shunt routes in the living skin must then penetrate the bulk stratum corneum in the diffusion apparatus, with consequent anomalies in relating the data to the in vivo situation.

Further investigation concerning enhanced percutaneous absorption of nifedipine will be reported and discussed elsewhere.

In summary, the superfluous thermodynamic potential of the drug substance in the volatile/nonvolatile solvent system has proved to be applicable for the driving force of enhanced transdermal delivery. An additional conclusion to be drawn from these results is that information obtained from in vitro diffusion studies with an artificial EVA membrane or excised skin can be used with some caution for assessing the in vivo availability from dermal formulations.

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REFERENCES


