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Transdermal absorption of nifedipine from microemulsions of lipophilic skin penetration enhancers

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Abstract

Oil/water microemulsions of six lipophilic skin penetration enhancers (oil of ylang ylang, lavender oil, cinnamon oil, cineole, menthone and menthol) containing ethanol as emulsifying agent were prepared. These systems were used as vehicles for the percutaneous absorption of nifedipine. The effect of microemulsions containing 50 and 25% ethanol on the percutaneous absorption of nifedipine were compared with formulations consisting of solutions of penetration enhancers in 100% ethanol. Microemulsions containing 50% ethanol were found to be superior vehicles for the percutaneous absorption of nifedipine. A probable mechanism by which microemulsions enhance the percutaneous absorption of drugs effectively is explained.

Keywords: Microemulsion; Penetration enhancer; Nifedipine; Stratum corneum

1. Introduction

Transdermal administration of drugs promises many advantages in therapy over oral or parenteral administrations (Chien et al., 1989; Friend et al., 1989; Seki et al., 1991). Absorption via the transdermal route is limited by the generally poor penetration of drugs through the stratum corneum, the outermost layer of the skin which comprises keratin-rich dead cells embedded in a complex lipid matrix. Reducing the barrier properties of the stratum corneum by using penetration enhancers is one method by which drug absorption can be improved (Sasaki et al., 1990; Barry, 1991). Vehicles play an important role in the percutaneous absorption of drugs (Nannipieri et al., 1990; Loth, 1991). Recently, particulate systems, like liposomes and nanoparticles, have been investigated as vehicles for the transdermal absorption of drugs (Knepp et al., 1990; Cappel and Kreuter, 1991; Lasch et al., 1991). For several years, microemulsions have been studied in pharmaceutical applications (Gallarate et al., 1988; Iwamoto et al., 1991). Microemulsions are characterized as thermodynamically stable, clear or slightly opalescent isotropic systems. They are self-emulsifying, possess low viscosity and consist of an aqueous compound, a lipophilic compound and an emulsifying agent. Currently, microemulsions have been recognized as good vehicles for the percutaneous absorption of drugs (Fevrier et

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al., 1991; Kemken et al., 1991, 1992). Unlike liposomes, microemulsions are relatively stable at room temperature for a long period of time and can solubilize a considerable amount of hydrophobic drugs in their lipophilic domain.

Oil/water microemulsions of six lipophilic penetration enhancers: lavender oil, oil of ylang ylang, cinnamon oil, cineole, menthone and menthol containing ethyl alcohol in different concentrations as the emulsifying agent were prepared and used as vehicles for nifedipine (a calcium channel blocker, which is widely used as an antihypertensive drug). The percutaneous nifedipine absorption promoting activities of these formulations were compared with true solutions of the penetration enhancers in absolute ethanol. Rabbit pinna skins were used for the permeability studies. Microemulsions containing 50% ethanol were found to be superior in promoting the percutaneous absorption of nifedipine.

2. Materials and methods

2.1. Materials

Nifedipine (Medinex Laboratory, India), cineole, menthone and menthol (Aldrich Chemical Co., U.S.A.) were used as obtained. Pharma grade lavender oil, oil of ylang ylang and cinnamon oil were purchased locally. All other chemicals used were of analytical grade.

2.2. Preparation of microemulsions

An excess amount of nifedipine was added to 50% aqueous ethanol in a stoppered conical flask and stirred continuously for 48 h. The suspension was then centrifuged and the clear supernatant saturated with the drug was separated. Penetration enhancer was added to this drug solution to a final concentration of 5% (v/v) and thoroughly mixed over a vortex mixer for 30 min to form a slightly opalescent microemulsion. In this way microemulsions containing oil of ylang ylang, cinnamon oil, lavender oil, menthol, menthone and cineole (vehicle system-1) were prepared. Similarly microemulsions containing 25% ethanol as emulsifying agent (vehicle system-2) were also prepared. Microemulsions of vehicle system-2 were less stable and of slightly larger particle size than microemulsions of vehicle system-1. This may be due to a slightly greater interfacial tension owing to the low alcohol concentration in vehicle system-2.

Formulations consisting of solutions of penetration enhancers in 100% ethanol saturated with nifedipine (vehicle system-3) were also prepared. The concentrations of penetration enhancers were the same in all preparations. Since nifedipine is light sensitive, all glassware used in the preparation process was wrapped in black paper. Extreme caution must be taken to avoid exposure of the contents to light at each step in the preparation process.

2.3. Preparation of rabbit pinna skin

Rabbit pinna skin is located on the inner side of the rabbit ear. The animals were killed by decapitation and the ears were removed immediately. An incision was made along the ear margin and the pinna skin was peeled away from the underlying cartilage. Skin was freed of any subcutaneous fat. Skins were washed with physiological saline and used for permeation studies.

2.4. In vitro skin permeation studies

All in vitro skin permeation studies were carried out in static glass diffusion cells (6 ml half cell volume and 1.13 cm^2 area of diffusion). Skin tissue with the stratum corneum facing the donor side was mounted carefully between the two half cells of the diffusion cell and fastened with a rigid clamp. The drug formulation was placed in the donor compartment and the receiver compartment contained pure 40% aqueous ethanol. The whole assembly was kept in a water trough over a magnetic stirrer/heater. Both the donor and receiver compartments were stirred continuously at controlled speed and the temperature was maintained at 37°C throughout the experiment. At predetermined times the entire contents of the receiver compartment were withdrawn and refilled with fresh solvent. The samples were as-

sayed for nifedipine spectrophotometrically at 337.5 nm using a Shimadzu UV-2100S instrument. Diffusion cells and all glassware used in this study were wrapped in black paper to avoid photodecomposition of nifedipine.

2.5. Statistical evaluation

Statistical evaluation of the experimental results was performed by two-way analysis of variance (ANOVA) test to elucidate the influence of different vehicle systems and penetration enhancers on the percutaneous flux of nifedipine.

3. Results and discussion

Recently, the influence of ethanol in aqueous solutions upon the transport behavior of several permeants across the skin has been investigated (Obata et al., 1991; Takayama et al., 1991). It has been reported that at higher concentrations of ethanol, there may be altered or additional pore/polar pathways formed in the stratum corneum as a result of a combination of changes in protein conformation, reorganization within the lipid polar head region or lipid extraction. Moreover, it has been observed that at 100% ethanol concentration the stratum corneum behaves as a purely porous membrane barrier unable to distinguish between highly lipophilic or polar permeants (Ghanem et al., 1992). Thus, vehicles consisting of 100% ethanol are expected to enhance the drug permeability of the skin to a greater extent than those containing lower concentrations of ethanol.

In the present investigation, it has been observed that the percutaneous nifedipine flux obtained with microemulsions containing 50% aqueous ethanol was much higher as compared to that with formulations consisting of 100% ethanol. Fig. 1–6 show the cumulative amount of nifedipine permeated through unit area of the skin from formulations consisting of different vehicle-enhancer combinations. Maximum drug permeation was achieved with formulations of vehicle system-1 and the efficiency of each formulation in the percutaneous absorption enhancement of nifedipine was found to depend on the type of penetration enhancer it contained. Among all the formulations, the one containing cinnamon oil was found to be the most efficient.

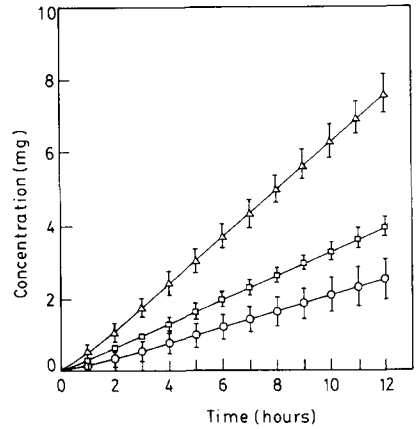

Fig. 1. Cumulative amount of nifedipine permeated through unit area of rabbit pinna skin from different vehicle systems containing cinnamon oil. Vehicle system-1 (△), vehicle system-2 (□), vehicle system-3 (○).

As compared to vehicle system-1, formulations of vehicle system-2 were less efficient in promoting the percutaneous absorption of nifedipine and that achieved with formulations of vehicle system-3 was found to be lower than that achieved with formulations of either vehicle system-1 or vehicle system-2. According to ANOVA test re-

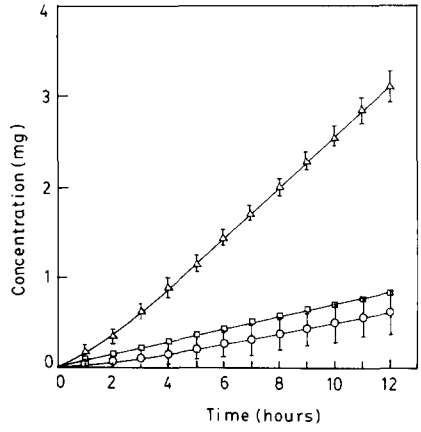

Fig. 2. Cumulative amount of nifedipine permeated through unit area of rabbit pinna skin from different vehicle systems containing lavender oil. Vehicle system-1 (△), vehicle system-2 (□), vehicle system-3 (○).

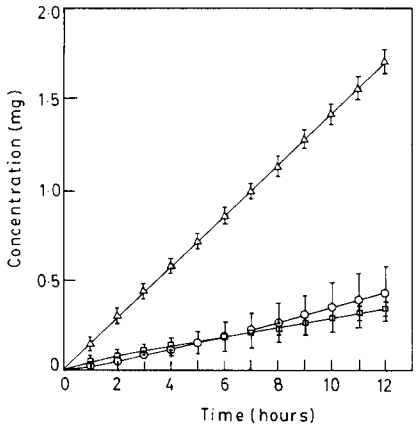

Fig. 3. Cumulative amount of nifedipine permeated through unit area of rabbit pinna skin from different vehicle systems containing oil of ylang ylang. Vehicle system-1 (△), vehicle system-2 (□), vehicle system-3 (○).

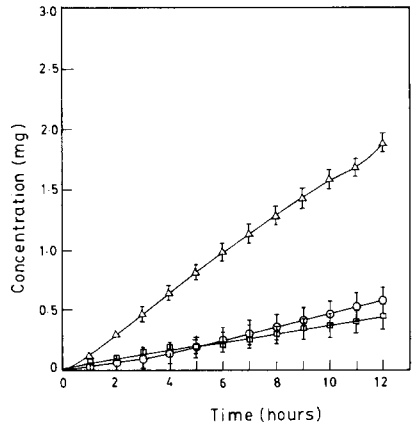

Fig. 5. Cumulative amount of nifedipine permeated through unit area of rabbit pinna skin from different vehicle systems containing menthone. Vehicle system-1 (△), vehicle system-2 (□), vehicle system-3 (○).

sults (Table 1), the differences between formulations of vehicle systems with respect to the percutaneous absorption of nifedipine was found to be highly significant. These results clearly indicated that penetration enhancers when used as oil/water microemulsions are more efficient than their solutions in the percutaneous absorption enhancement of nifedipine.

To explain a probable mechanism by which microemulsions enhance the percutaneous absorption of drugs efficiently, the histological and histochemical structure of the stratum corneum must to be taken into consideration.

The stratum corneum is the rate-limiting factor in the percutaneous absorption of drugs. Any significant enhancement in the skin permeability to drugs caused by either vehicles or by penetration enhancers is mainly due to a temporary distortion of the stratum corneum structure. Drug can permeate through two micropathways in the stratum corneum. One is the intercellular route and other is the transcellualr route. Among these

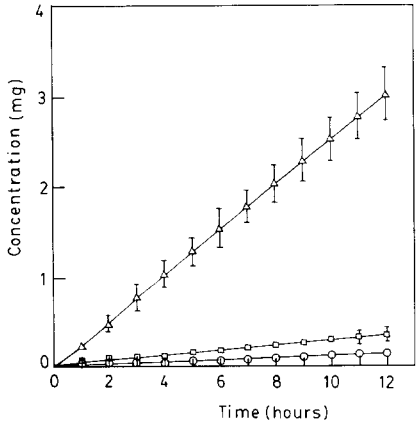

Fig. 4. Cumulative amount of nifedipine permeated through unit area of rabbit pinna skin from different vehicle systems containing cineole. Vehicle system-1 (△), vehicle system-2 (□), vehicle system-3 (○).

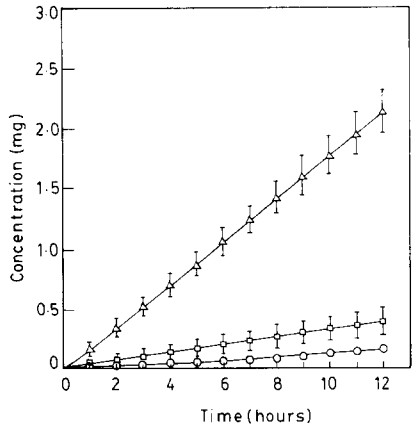

Fig. 6. Cumulative amount of nifedipine permeated through unit area of rabbit pinna skin from different vehicle systems containing menthol. Vehicle system-1 (△), vehicle system-2 (□), vehicle system-3 (○).

Table 1
Analysis of variance results

Source of variation	S.S.	D.F.	M.S.	F
Between vehicle systems	0.2381	2	0.11905	25.88
Between enhancers	0.224	5	0.0448	9.74
Residual	0.0461	10	0.0046	

S.S., sum of squares; D.F., degrees of freedom; M.S., mean square; F, variance ratio.

routes, the intercellular route plays a major role in the percutaneous uptake of drugs.

The molecular composition and reorganization of the intercellular route (region between the corneocytes) is still not completely defined. It is known that a complex mixture of essentially neutral lipids are arranged as bilayers with their hydrophobic chains facing each other, forming a hydrophobic bimolecular leaflet. Most of the lipophilic drugs pass through this region and it is called the lipid pathway. Polar head groups of lipids face an aqueous region forming a polar route. Hydrophilic drugs generally prefer this route. Recent findings indicate that small amounts of lipids are immobilized via covalent bonding to corneocyte surface proteins. Moreover, proteins have also been detected in the intercellular spaces (Barry, 1991).

A dermally applied microemulsion is expected to penetrate the stratum corneum and to exist intact in the whole horny layer, since it has been proved by double-labelling studies (Hofland et al., 1990) and freeze-fracture electron microscopic studies (Weiner and Egbaria, 1990) that dermally applied liposomes, which resemble microemulsions, exist intact in the whole horny layer of the skin. Once it enters into the stratum corneum, the microemulsion can simultaneously alter both the lipid and the polar pathways. The lipophilic domain of the microemulsion can interact with the stratum corneum in many ways. The drug dissolved in the lipid domain of a microemulsion can directly partition into the lipids of the stratum corneum or the lipid vesicles themselves can intercalate between the lipid chains of the stratum corneum, thereby destabilizing its bilayer structure. In effect, these interactions will lead to increased permeability of the lipid pathway to the drugs.

On the other hand, the hydrophilic domain of the microemulsion can hydrate the stratum corneum to a greater extent. There is a general experience that hydration of the skin plays an important role in the percutaneous uptake of drugs. When the aqueous fluid of the microemulsion enters the polar pathway, it will increase the interlamellar volume of stratum corneum lipid bilayers, resulting in the disruption of its interfacial structure. Since some lipid chains are covalently attached to corneocytes, hydration of these proteins will also lead to the disorder of lipid bilayers. Similarly, swelling of the intercellular proteins may also disturb the lipid bilayers; a lipophilic penetrant like nifedipine can then permeate more easily through the lipid pathway of the stratum corneum. The greater drug penetration enhancing activity of microemulsions as compared to vehicles consisting of true solutions may be attributed to the combined effect of both the lipophilic and hydrophilic domains of microemulsions.

The particle size of the microemulsion may also affect its efficiency in promoting the percutaneous absorption of drugs. When the particle size is very small, there is a chance that the number of vesicles that can interact on a fixed area of stratum corneum will be increased, thereby increasing its efficiency in the percutaneous uptake of drugs. On the other hand, if the particle size of microemulsion is greater, not only does the number of vesicles that can interact on a unit area of the skin decrease but the penetration of the vesicles into the skin also becomes more difficult. As a result, the efficiency of the vehicle is reduced. This may be the reason why microemulsions of vehicle system-2, whose particle size is larger than that of vehicle system-1, showed poor drug penetration enhancing activity.

4. Conclusion

Vehicles play an important role in the percutaneous absorption of nifedipine. Formulations consisting of oil/water microemulsions of lipophilic skin penetration enhancers were found to be superior vehicles for nifedipine as compared

to formulations consisting of true solutions of penetration enhancers. This superiority of microemulsions over other vehicles may be attributed to the combined effect of both the lipid and the aqueous phases of the microemulsions. Lipophilic penetration enhancers could be used as oil/water microemulsions for topical applications so that the penetration enhancing activity of these agents together with the influence of microemulsions could act synergistically to accelerate the percutaneous absorption of drugs.

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