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ENHANCEMENT EFFECTS IN THE PERCUTANEOUS ABSORPTION OF ALPRAZOLAM THROUGH HUMAN SKIN IN VITRO

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ABSTRACT

The enhancement effects of some chemicals on the percutaneous absorption of alprazolam through human skin was investigated in vitro. Linoleic acid, oleic acid, Comperlan F® (linoleic acid diethanolamide), Comperlan KD® (coconut fatty acid diethanolamide) and Ethomeen C12® (bis-(2-hydroxyethyl)cocamine) were evaluated for their enhancing effect either as neat solvents or combined with propylene glycol. The effects of skin pretreatment with the enhancers on the percutaneous absorption of alprazolam from a drug suspension in an aqueous gel were also investigated: skin pretreatment with some enhancers has shown potentially interesting aspects. The determination of alprazolam partitioning into untreated and pretreated horny layer supported the idea that, with oleic and linoleic acid, a contribution to the modification of the diffusional resistance of the skin was given by an increase of the drug solubility in the stratum corneum.

INTRODUCTION

In a previous paper a preliminary study was reported whose objectives were to determine the enhancement effects of some chemicals on the permeation of alprazolam, an anxiolytic, antidepressant agent, through hairless mouse skin in vitro (1). Although hairless mouse skin is generally more responsive to enhancer effects than human skin, nevertheless preliminary studies with animal skin enable researchers to select the more promising enhancers and, hence, to make reasoned use of human epidermis, a rare material whose removal from human cadavers is not allowed by local legislation in many countries. In the above mentioned paper (1), interesting results were obtained with a linoleic acid/propylene glycol binary mixture, Ethomeen C12® (bis-(2-hydroxyethyl)-

cocamine), Comperlan F® (linoleic acid diethanolamide), and Comperlan KD® (coconut fatty acid diethanolamide). The results encouraged us to carry on the study of percutaneous absorption of alprazolam using human skin, whereby more relevant data may be obtained.

MATERIALS

Upjohn SpA (Milano, Italy) generously provided alprazolam (8-chloro-1-methyl-6-phenyl-4H[1,2,4]triazolo[4,3-a][1,4]benzodiazepine). Oleic acid (OLA) and propylene glycol (PG) were purchased from E. Merck (Darmstadt, Germany). Comperlan F® (linoleic acid diethanolamide), and Comperlan KD® (coconut fatty acid diethanolamide) were supplied by Henkel HGaA (Dusseldorf, Germany). Ethomeen C12® (bis-(2-hydroxyethyl)cocamine) was obtained by Akzo Chemie Italia. Linoleic acid (LNA) was purchased from Sigma Chem. Co. (St. Louis., MO, USA). Tegiloxan® (polydimethylsiloxane oils, Tegiloxan 30,000®) was a gift from Tego Italiana S.r.l. (Milano, Italy). Sodium carboxymethylcellulose (Tylose C®) was obtained by Hoechst Italiana (Milano, Italy). The aqueous gel (GEL) consisted of 3% (w/w) sodium carboxymethylcellulose in normal saline containing 0.035% formaldehyde.

Human skin was obtained by operation for cosmetic surgery. The epidermal layer was removed from the dermis after immersion of the whole skin in water at 60°C for 30 sec. The epidermal sheets were dried in a desiccator and subsequently stored at 4°C. They were rehydrated by immersion in water prior to use.

Strips of human callus were removed from the plantar surface of volunteers with a scalpel, and stored in a desiccator over $CaCl_2$

EXPERIMENTAL PROCEDURES

Percutaneous penetration experiments - The diffusion rates of alprazolam across the excised epidermis were measured using diffusion cells based on the Franz design (2), having 1.13 cm^2 cross sectional area. The experiments were carried out at 30°C. Substantially the same procedure as that described previously for both untreated and pretreated skin was adopted, with few changes (1). In brief, the receptor phase was 8 ml of normal saline containing 0.035% formaldehyde as preservative (NSF) (3), whereas the donor phase consisted of a 0.2 ml (or 0.3 g, for semisolid preparations) aliquot of a drug suspension in the test vehicle. Pseudosink conditions were maintained throughout the experiments. The total amount of the drug penetrating through the unit membrane surface and into the receptor was determined and plotted as a function of time. Comparison between the percutaneous penetration of alprazolam from the vehicles under study was based on flux values, expressed as the mass of drug passing across 1.0 cm^2 of skin per unit time; drug flux was calculated as described previously (4). Comparisons between alprazolam suspensions in the vehicles under study were made with epidermal sheets obtained from the same skin sample; flux values relative to a reference vehicle were calculated and averaged. For the permeability measurements through pretreated skin, the untreated epidermal sheet (reference) was floated on water for 15 hr before the start of the permeation experiment and that selected for the

pretreatment was, in the meanwhile, treated with the pretreating vehicle. As a rule permeation through both the pretreated epidermal sheet and the reference sheet was investigated using alprazolam suspension in GEL, even though a drug suspension in Tegiloxan® was occasionally employed. Mean relative flux values are shown in Tables 1 and 2.

Enzymatic degradation - The possibility of enzymatic degradation of alprazolam during skin permeation experiments was assessed according to a procedure described in a previous paper (1). Since no degradation products resulted from both thin-layer chromatographic and gas chromatographic analyses of the receptor phase of permeation experiments, alprazolam was assumed to diffuse unchanged across skin in vitro.

Human callus/NSF partition coefficients - Fragments of human callus were hydrated by exposure to water vapour in a closed system at 37°C until the water content was about 50% of the dry tissue. Some hydrated fragments (0.1 g dry weight) were subsequently treated with the enhancers by immersing the tissue in the proper vehicle for 48 hr at 30°C ; then, prior to partition experiment, the vehicle was carefully removed by filter paper. Both treated and untreated tissues were equilibrated with 5.0 ml of alprazolam solution in NSF according to the following procedure. The tissue was placed in a screw-cap vial and added with the drug solution. The vial was gently tumbled from time to time at 30°C. The equilibrium concentration of the aqueous phase was determined after 24 hr; missing drug was assumed to have entered the tissue. The dry weight of the tissue was used for calculating the partition coefficient, which was expressed as the ratio of molal to molar concentrations.

Assay method - Gas chromatographic analysis: Alprazolam concentration in aqueous samples was measured by a gas chromatographic analysis according to a procedure described previously (1). Thin-layer chromatography: This analysis was accomplished according to a procedure described by Wouters et al. (5). Ready made plates (DC - Fertigplatten Kieselgel 60 F 254; E. Merck, Darmstadt, Germany) were used as received. Paper-lined chromatographic chamber was equilibrated with the mobile phase at least 1 hr. Drug amounts of about 2 μg were spotted on the chromatoplate. An ethyl acetate/methanol/25% ammonia mixture was used as mobile phase. The plate was developed over a distance of 15-17 cm, dried in a stream of warm air and examined under ultraviolet lamp having a maximum output at about 254 nm.

RESULTS

Since drug saturated vehicles were used, the thermodynamic activity of alprazolam did not change with varying vehicle. Then, as the skin is supposed to provide the only barrier to drug penetration, flux changes resulting from changes in vehicle composition were considered indicative of alteration of skin permeability caused by a direct action of the vehicle on skin.

When the amounts of alprazolam permeated from neat vehicles through the unit area of skin were plotted *vs* time, straight lines following a lag time were obtained with LNA,

TABLE 1

Permeation Data for Alprazolam through Untreated Skin

Vehicle	N [a]	Relative Flux [b]
LNA	11	1 [c]
LNA/PG	3	8.50 ± 1.91
OLA	6	1.36 ± 0.39
OLA/PG	4	7.30 ± 1.75
Comperlan F®/PG (7.5:2.5)	4	8.75 ± 1.50
Comperlan F®/PG (5:5)	3	6.10 ± 1.80
Comperlan F®/PG (2.5:7.5)	3	3.71 ± 0.92
PG	5	1.21 ± 0.29
GEL	4	0.27 ± 0.11

[a] Number of determinations. [b] Mean flux relative to LNA±standard deviation. [c] The mean flux from LNA was 0.44±0.20 $\mu g \cdot cm^{-2} \cdot hr^{-1}$.

TABLE 2

Permeation Data of Alprazolam through Skin Pretreated with Selected Vehicles

Pretreatment Vehicle	Permeation Vehicle	Relative Flux [a]
none	GEL	1
LNA	GEL	3.3, 9.0, 5.7
LNA/PG	GEL	5.9, 10.5
OLA	Gel	4.6, 10.5
OLA/PG	GEL	6.0, 4.5, 15.0
Ethomeen C12®	Gel	24, 28 [b]
	Tegiloxan®	22

[a] The reference flux was obtained by a permeation through untreated skin from an alprazolam suspension in GEL. Each reported value corresponds to a single run. [b] The steady-state flux was not reached; the reported fluxes are mean values calculated in the range 30-60 hr (see Fig. 1).

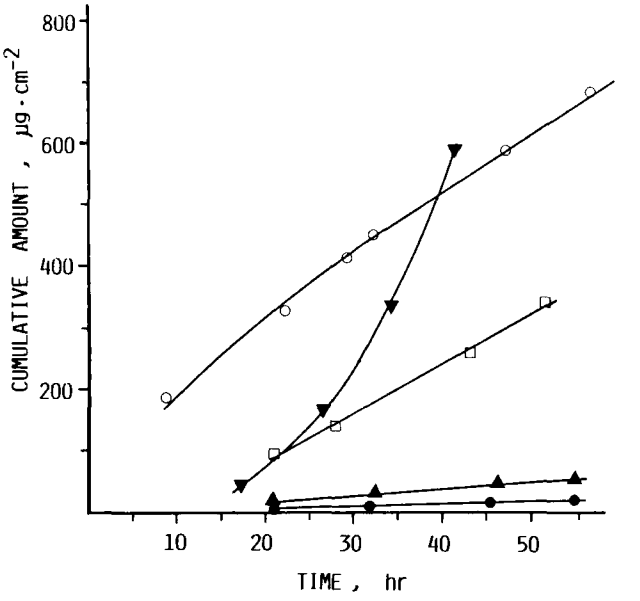

FIGURE 1

Typical permeation curves from a drug suspension in GEL (● , ○), LNA (▲), Tegiloxan® (□) or Ethomeen C12® (▼) through untreated skin (closed symbols) or skin pretreated with Ethomeen C12® (open symbols). The run was carried out with five sheets from the same skin sample.

OLA, PG and GEL. The steady-state flux data from these vehicles are reported in Table 1. Inspection of the table shows that the flux data from OLA and PG were not significantly different from that from LNA whereas drug flux from GEL was about four times lower than that from the reference vehicle. The lag time values were grater than 10 hr for PG, whereas they were never greater than 10 hr for both acids and GEL. Permeation curves showing a non-linear pattern were obtained with Ethomeen C12®; consequently, the relative-flux values were not calculated. Nevertheless, Ethomeen C12® was more effective than LNA in improving permeation of the drug since the initial times of the experiments. A typical permeation curve from a drug suspension in Ethomeen C12® is shown in Fig. 1.

Results obtained with the amides Comperlan F® and Comperlan KD® have not been reported because of both their scarce enhancing effect and scarcely reproducible results. Nevertheless, when Comperlan F® was added with PG, reproducible steady-state fluxes were obtained. The relative flux values, which are listed in Table 1, indicate that Comperlan F®/PG mixtures were more effective than LNA in promoting alprazolam permeation. Unfortunately, the corresponding lag times were very high (16 - 20 hr). When LNA or OLA were saturated with 15% PG the flux values from the resulting

TABLE 3

Partition Coefficient Data for Alprazolam

Pretreatment Vehicle	Partition Coefficient	
	Kc [a] ($ml \cdot g^{-1}$)	Ko [b]
none	17.5 ± 0.5	132
LNA	58.3 ± 6.5	
LNA/PG	60.1 ± 7.3	
OLA	45.9 ± 4.1	
OLA/PG	47.5 ± 5.1	
Ethomeen C12®	24.9 ± 1.9	

[a] Human callus/NSF partition coefficient; mean ± standard deviation (N = 3). The equilibrium concentrations in NSF were about 50% of the saturation concentration. [b] n-Octanol/NSF partition coefficient (Ref. 1).

vehicles were similar to those from Comperlan F®/PG (7.5:2.5), i.e. about 8-fold over that from LNA; however, both LNA/PG and OLA/PG did not substantially modify the lag-time values which had been observed for neat vehicles. Conversely, when PG was added to Comperlan KD® or Ethomeen C12®, the performances of the resulting vehicles did not improve over those observed for the neat compounds.

LNA, OLA, their mixtures with PG, and Ethomeen C12® were selected for the pretreatment of the skin. Permeation data through pretreated skin were compared with those through untreated skin from an alprazolam suspension in GEL. The permeation profiles obtained with skin pretreated with LNA, OLA or their mixtures with PG showed a linear pattern following a definite lapse of time. Inspection of the permeation data reported in Table 2, shows that alprazolam flux from GEL was improved by skin pretreatment. Nevertheless, the lag-time values were not substantially different from those through untreated skin from GEL. A different behaviour was observed when the skin was pretreated with Ethomeen C12®; indeed, the permeation profiles from GEL showed a pattern similar to that previously observed with hairless mouse skin (1), i.e., the profiles tended towards a linear pattern after an initial burst effect (Fig. 1). Consequently, the relative-flux values, 24 and 28, reported in Table 2 for the pretreatment with Ethomeen C12® were calculated at the end of the permeation, in the range 30-60 hr.

In Table 3, alprazolam partition coefficients, Kc, between pretreated or untreated human callus and NSF, are shown; the n-octanol/NSF partition coefficient , Ko, is also reported.

DISCUSSION

The stratum corneum is a heterogeneous barrier which is generally treated as a homogeneous membrane across which solute flux is assumed to be directly proportional to the stratum corneum/vehicle partition coefficient and solute diffusivity in the stratum corneum and inversely proportional to the membrane thickness. Nevertheless, relatively few measurements of stratum corneum/vehicle partition coefficient have been reported in the literature. The scarce availability of the tissue and the large amounts usually requested for the experiments have presumably discouraged the researchers from accomplishing such determinations. Although some components of human callus may differ from those of human stratum corneum, the structures of the two tissues are not substantially different; indeed, both tissues consist of a mosaic of keratinized cells distributed in an intercellular lipid domain (6). It follows that, although the absolute values of Kc, reported in Table 3, may be somewhat different from the true stratum corneum/NSF partition coefficients, the Kc variations presumably reflect the corresponding variations in the stratum corneum/NSF partition coefficients.

A comparison of the flux and Kc values, reported in Tables 2 and 3, respectively, reveals that the pretreatment of the epidermal sheets and the callus with LNA, OLA or their mixtures with PG increased both alprazolam flux and partition coefficient. Although LNA/PG and OLA/PG mixtures were about 8 times as effective as their neat components in promoting alprazolam permeation through untreated skin (Table 1), the flux values obtained from the pretreatment of the human skin with such mixtures were not significantly different from the corresponding fluxes measured with skin pretreated with neat LNA or OLA (Table 2). The apparent contradiction of these results is solved by the following considerations. In the permeation experiments through untreated skin, with an acid/PG mixture as the donor phase, the vehicle supplied the skin continuously with PG and the acid, so both chemicals were present within the tissue throughout the experiment. On the other hand, in the experiments through pretreated skin the pretreating acid/PG mixture was removed from the skin surface by a flow of water, after 15-hr pretreatment and before the start of the permeation; this treatment caused a PG depletion and/or dilution. So, the above results may be reconciled by admitting a leak of PG from the pretreated tissue. This hypothesis agrees with the Kc data reported in Table 3; indeed, owing to PG leakage from the tissue in the course of the experiment, the Kc values obtained from the pretreatment of the human callus with LNA, OLA or their acid/PG mixtures were not significantly different from one another. In conclusion, the aforesaid observations suggest that a) LNA and OLA increase the drug partitioning into the stratum corneum and, as a consequence, contribute to reduce the diffusional resistance of the skin; b) the enhancing effects of the acids still remain after the removal of the pretreating vehicle; c) PG-skin interaction is promptly reversible.

As shown in Fig. 1, Ethomeen C12® had an effect on the barrier function of the skin exceedingly time dependent. However, the high potential of this chemical as an enhancer could be well utilized for the pretreatment of the skin. In fact, although alprazolam flux through skin pretreated with Ethomeen C12® from a drug suspension in GEL (Table 2) was not higher than the fluxes through untreated skin from alprazolam suspensions in LNA/PG or OLA/PG (Table 1), nevertheless the application of a drug suspension in a non-irritant vehicle, such as the aqueous gel, GEL, on skin pretreated for few hours with Ethomeen C12® is by far more advantageous than a prolonged

contact of the skin with more irritant vehicles. According to some authors (7), OLA itself can cause severe skin irritation.

The pattern shown in Fig. 1 for the permeation from GEL through the skin pretreated with Ethomeen C12® might find an explanation in a slow and partial recovery of the skin properties consequent on a slow diffusion of Ethomeen C12® in and out of the skin in the course of the permeation. This hypothesis is supported by the partitioning data of alprazolam into human callus (Table 3). Indeed, the partition coefficient of alprazolam between the human callus pretreated with Ethomeen C12® and NSF is only moderately higher than that between the untreated callus and NSF. The hypothesis of the barrier function of skin being disrupted by the pretreatment with Ethomeen C12® and, hence, the pattern shown in Fig. 1 being a measure of the drug diffusion in the applied gel were ruled out as a burst effect similar to that seen with GEL was observed in a separate experiment where a drug suspension in water was applied to epidermis pretreated with Ethomeen C12®. Indeed, in the cell, the solid drug rapidly settled over the epidermis surface, thus ruling out the possibility of concentration gradients forming within the vehicle. Interestingly, quite a different permeation profile, with no burst effect, was obtained from a single run where a drug suspension in polydimethylsiloxane oil, Tegiloxan®, instead of in GEL, was applied to the pretreated skin (Fig. 1); the steady-state flux was practically identical to that obtained from alprazolam suspensions in GEL (Table 2), but a lag time was present. The very low solubility of alprazolam in Tegiloxan® ($0.7 \cdot 10^{-2}$ mg/ml) may play a role in lengthening the time for attainment of the steady-state conditions. The result is of some practical interest in that silicones are used as pressure-sensitive adhesives, cosolvents or liquid excipients in transdermal therapeutic systems (8).

In conclusion, the permeation data for alprazolam through human epidermis support some results previously obtained with hairless mouse skin (1). The relative effectiveness of the binary mixtures OLA/PG and LNA/PG in promoting alprazolam flux has even appeared superior with human skin than with hairless mouse skin. Satisfactory results were obtained by pretreating the skin with the enhancers before application of a drug suspension in a non-irritant vehicle. For example, Ethomeen C12® might be utilized at best by pretreating the skin with the enhancer before the application on the tissue of an alprazolam suspension in GEL or Tegiloxan®; while the permeation profile changed with changing the vehicle, the steady-state flux was practically unchanged. The enhancing effects obtained by pretreating the skin with either neat compounds OLA and LNA or their mixtures with PG were not different from each other. This result was explained by assuming PG to diffuse in and out of the skin easily and both LNA and OLA to bind to the skin strongly. The determination of alprazolam partitioning into untreated and pretreated human callus supported the idea that, with OLA and LNA, a contribution to the modification of the diffusional resistance of the skin was given by an increase of the drug solubility in the stratum corneum. It is well known that OLA may act by modifying the diffusivity of the penetrants in the stratum corneum (9-11). Although the present experimental data are not suitable for an evaluation of the effect of the two acids on the apparent diffusivity of alprazolam in the stratum corneum, it is our opinion that the effects of OLA and LNA on the diffusivity and solubility of the drug are co-operative.

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