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Original Article

Stereoselective Permeation of Tretinoin and Isotretinoin through Enhancer-Treated Rat Skin. II. Effects of Lipophilic Penetration Enhnacers

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Abstract

Many properties of chemicals depend on their stereochemistry. Among these, the effects of stereoisomerism on percutaneous absorption of drugs are not well studied. We have previously shown that permeation of tretinoin and isotretinoin (two geometric isomers) through hydrophilic enhancers-treated rat skin is stereoselective. As, depending on their lipophilicity, penetration enhancers can change permeation pathway of drugs, it was decided here to investigate permeation of the same stereoisomers through lipophilic enhancer-treated skin.

Oleic acid, that mainly affects stratum corneum intercellular lipids, was chosen as the lipophilic penetration enhancer for this study. Permeation of retinoids through untreated and enhancer-treated rat skin was studied at room temperature. These studies employed static diffusion cells, saturated drugs in propylene glycol:water mixture (75:25, v/v) as the donor phase and aqueous solution of $Tween_{20}$ as the receptor phase.

Permeation of retinoids through untreated skin was too low to be detected in the receptor phase even by the used HPLC method with sensitivity of 10 ng ml^{-1}. Oleic acid increased the permabilities of retinoids to measurable values. In oleic acid-treated skin, tretinoin permeated by about 1.3 times faster than isotretinoin ($P<0.0001$). However, permeability coefficients, that are concentration-normalized fluxes, showed a reverse order. Permeability coefficient of isotretinoin through the same membrane was significantly ($P=0.0002$) higher than that of tretinoin. On the other hand, the difference between permeation lag-times of retinoids was not that significant ($P=0.062$). These data show that stereoselctive permeation of these retinoids through oleic acid-treated rat skin is mainly a partitioning-related phenomenon. Diffusion coefficient might play only a minor role.

Present results are partly different from those of our previous study on hydrophilic enhancers and clearly show that the type of enhancer and its mode of action can affect stereoselective permeation of drugs through the skin.

Keywords: Skin; Permeation; Stereoselective; Tretinoin; Isotretinoin; Enhancers; Oleic acid.

Introduction

Stereoselectivity has been frequently reported in pharmacokinetic and pharmaco-dynamic studies with isomeric drugs. Permeation of drugs through biological membranes can also be stereoselective. It has been shown that permeation of L- and D- lactic acid and S- and R-mandelic acid through intestinal epithelial cells is stereoselective and that this phenomenon is pH and concentration dependent (1).

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There are also a few reports regarding stereoselective percutaneous absorption of drugs. Diastromers of isosorbide dinitrate show stereoselective percutaneous absorption (2). It has also been shown that tretinoin permeates human skin faster than its geometric isomer, isotretinoin (3). Stereoselective percutaneous absorption of drugs is not always due to stereoselective permeation. It is sometimes due to stereoselective enzymatic degradation in the skin as shown for propranolol prodrugs (4).

Unfortunately, many drugs do not permeate the skin well enough to be suitable for a transdermal formulation. Different methods, including application of chemical penetration enhancers, have been used to overcome this problem (5, 6). Chemical penetration enhancers can alter the properties of permeation pathway (7). Therefore, stereoselective permeation of drugs through normal skin cannot be easily extrapolated to the enhancer-treated membrane.

In agreement with the above discussion, we have shown that permeation of tretinoin and isotretinoin through enhancer-treated rat skin is stereoselective and that the type and concentration of enhancer affect this phenomenon (8). Tretinoin (all-trans retinoic acid, Figure 1) and isotretinoin (13-cis retinoic acid, Figure 1) are geometric isomers and both are available as topical anti-acne formulations (9).

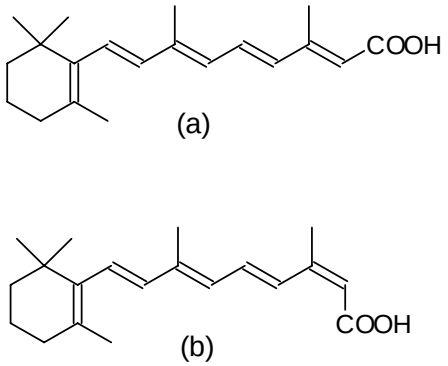

Figure 1. Chemical structures of tretinoin (a) and isotretinoin (b).

Chemicals permeate the skin mainly through the intact epidermis (transepidermal pathway) in which the stratum corneum provides the major permeation barrier (10). There are two pathways (intercellular and transcellular) available for permeation of drugs across the stratum corneum (Figure 2). The intercellular pathway, which is considered as the main route for permeation of most drugs, is filled with a lipid-based lamellar liquid crystalline structure (7, 10, 11). The transcellular pathway includes protein-filled cell cytoplasm and a protein-lipid cellular envelope (10).

Depending on their lipophilicity and mode of action, enhancers can change permeation pathway of drugs, for example from intercellular to transcellular pathway, as shown

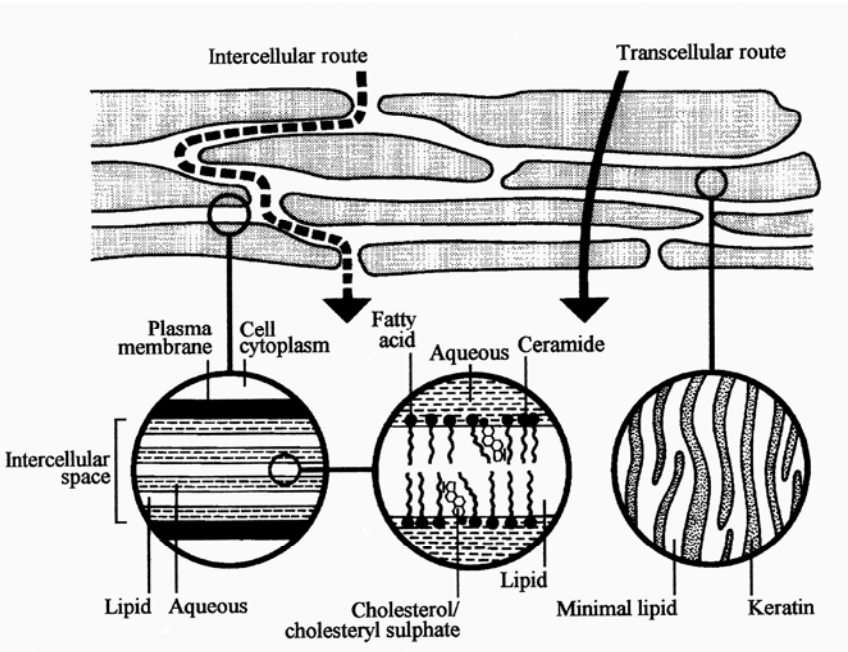

Figure 2. Schematic representation of stratum corneum and its intercellular and transcellular pathways of drug permeation. From Reference 10 with permission.

in stratum corneum models (11). Our previous stereoselectivity study used sodium dodecyl sulphate (SDS) and ethanol as the penetration enhancers (8). Ethanol and SDS are hydrophilic. SDS mainly affects the transcellular pathway and the level of hydration of the stratum corneum (12). It is also active on stratum corneum intercellular pathway as shown in stratum corneum models (13). Ethanol has a mixed effect on stratum corneum barrier and depending on its concentration, it can affect stratum corneum level of hydration, disrupt intercellular lipids or cause conformational change within the keratinized protein component of keratinocytes (14).

It was decided here to investigate the effects of a different and very important class of enhancers, lipophilic intercellular lipid-disturbing, on stereoselective permeation of tretinoin and isotretinoin. Oleic acid, which is lipophilic and mainly affects SC intercellular lipids and has been shown to be a lipid fluidizer (15), was chosen as the penetration enhancer for this study.

Experimental

Materials

Tretinoin and isotretinoin (reference standard) were supplied by Roche (Switzerland) and Sigma (USA), respectively. $Tween_{20}$, oleic acid, and propylene glycol (99%) were purchased from Merck (Germany). All materials were used as received.

Skin permeation studies

Preparation of skin

Abdominal skin from young male rats was used for this study. Rats were first sacrificed by placing them in a chloroform-saturated chamber. Abdominal hairs were then cut by an electrical hair clipper and full-thickness skin was separated surgically. The separated skin was cleaned from subcutaneous fat, muscle and vasculature and kept frozen at –20°C until use.

General procedure

For each experiment, skin samples were defrosted and sandwiched between donor and receptor chambers of home-made Franz-type diffusion cells (effective surface area of 5 cm^2), while epidermis faced the donor compartment. The receptor chamber was then filled with 30 ml receptor phase and the donor chamber with 4 ml of either oleic acid or control solvent. The system was then stored at room temperature for 12 h to allow enhancer treatment and skin equilibration with the receptor phase. After this period of time, the contents of both donor and receptor chambers were removed. The receptor chamber was then washed twice and filled with 30 ml fresh receptor phase. Four ml of drug solution or its control solvent was placed into the donor compartment and this point was considered as ‘time zero’. As retinoids are photosensitive (3), diffusion cells and other glassware used for handling of retinoids were covered with aluminum foil to minimize photodegradation. All experiments were performed at standard laboratory temperature.

Serial sampling of the receptor phase was performed for 24 h and the amount of absorbed drug was measured. Cumulative amount of permeated drug was plotted against time and the slope of the linear part of the graph (permeation flux) was measured, from which permeability coefficient and lag-time were calculated (10). The differences between permeation parameters of tretinoin and isotretinoin were analyzed statistically using a two-tailed t-test analysis, assuming that data are distributed normally and the populations have equal variances.

Receptor and donor phases

Water or other aqueous systems are usually used as the receptor phase in skin permeation studies. Tretinoin and isotretinoin are practically insoluble in water (9). In such a condition, an aqueous phase containing co-solvents or solubilizers becomes necessary. To find a suitable receptor phase, we performed a preliminary permeation study with retinoids using different receptor phases. These receptor phases included propylene glycol aqueous solutions (25, 50 and 75%, v/v), $Tween_{20}$ aqueous solutions (0.5 and 1.0%, w/v) and water. Results showed that both Tween solutions can provide a perfect sink condition. To minimize the possible interactions of this nonionic

surfactant with skin, the lower concentration (0.5 % solution) was chosen as the receptor phase. Such a system is not expected to influence the barrier properties of the skin (16). Solubility of retinoids in this receptor phase was measured to be around 45 µg/ml.

Saturated solution of drugs in propylene glycol:water system (75:25, v/v) was used as the donor solution. Solubility of tretinoin and isotretinoin in the donor solution was measured to be 33 and 23 µg/ml, respectively.

Drug measurement

Drug determination in enhancer-treated skin was performed by UV spectrophotometric assay. UV spectrophotometric method was performed at 360 nm using Spectronic 601 (Milton-Roy, USA). Standard working curves were constructed from known concentrations of drugs in solutions.

To evaluate the possibility of release of chemicals from skin and interaction of these materials with the UV assay method, different control permeation studies were performed. These control studies used propylene glycol:water solution (75:25, v/v, without any drug) as the donor phase and were performed on both untreated and oleic acid pretreated skin samples. Results showed that there is no interference of skin materials with the UV method.

Accuracy of the above-mentioned UV method, in terms of quantitative measurement of retinoids in the receptor phase, was confirmed by an HPLC method described previously (8). The same HPLC method was used to measure the permeation of retinoids through intact skin (without enhancer treatment).

Effects of OA on isomerisation of drugs

Oleic acid was applied to the skin as pretreatment. In comparison to other methods, e.g. co-administration of drugs and enhancers, the pretreatment method provides decreased drug-enhancer contact throughout the course of the experiment, and therefore, minimizes the possibility of effects of enhancers on isomerisation of drugs (if any). However, the effects of oleic acid on isomerisation of drugs were studied. To study this effect, 2 ml oleic acid was first added to 2 ml of a 6 mg/ml tretinoin solution in propylene glycol:water mixture (75:25, v/v). Containers were then covered with aluminum foil and stored at room temperature. Retinoid content of the containers was then measured at the start and after 18 h by the HPLC method described previously (8). Results were then compared to those of similar systems containing either of propylene glycol:water mixture (75:25, v/v) or water as the controls. Results showed that there is no difference between isomer contents of enhancer-containing systems and those of the controls.

Results and Discussion

Permeation of retinoids through untreated skin

Permeation of tretinoin and isotretinoin through full-thickness rat skin in the absence of oleic acid was rather low and we were not able to detect the retinoids in the receptor phase even by the HPLC method with a sensibility of around 10 ng/ml. Using ^{14}C-labeled retinoids, Lehman et al. (3) measured permeation of tretinoin and isotretinoin through human and monkey skin. Their results showed fluxes of less than 0.1 ng cm^{-2} h^{-1} through human skin at maximum thermodynamic activities of tretinoin and isotretinoin. They also measured permeation flux of isotretinoin through monkey skin to be around 1 ng cm^{-2} h^{-1} (3). In such permeation rates, the amounts of retinoids in the receptor phases of our system would be 2-20 times less than the sensibility of the used HPLC method (10 ng/ml).

Permeation of retinoids through oleic acid-treated skin

Figure 3 shows permeation profiles of tretinoin and isotretinoin permeated through oleic acid-treated rat skin. Results (Table 1, Figure 3) show that at saturated concentration (maximum thermodynamic activity), tretinoin permeates oleic acid-treated skin 1.3 times faster than isotretinoin ($P<0.0001$). These

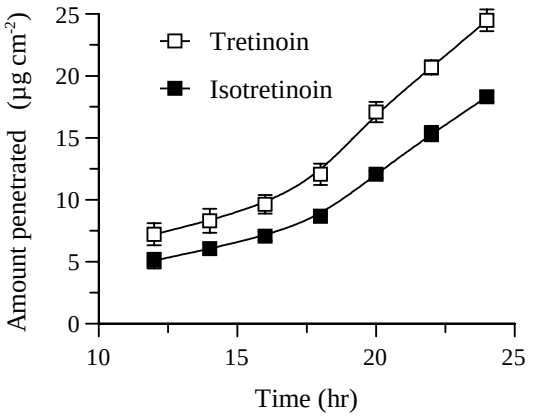

Figure 3. Cumulative amount of tretinoin and isotretinoin permeated through oleic acid-treated rat skin. Data are Mean ± SD, n=4.

results are in agreement with those reported by Lehman and co-workers who showed that the permeation flux (as measured according to drug concentration in the receptor phase) of tretinoin through untreated human skin was almost twice that of isotretinoin (3).

Lehman et al. (3) applied these retinoids as isopropyl alcohol solutions and evaporated the solvent after application to the skin. In such a condition, during the evaporation, thermodynamic activity of the drug increases until it reaches to its maximum value. Therefore, in terms of thermodynamic activity, our system and that of Lehman and his group should be the same.

The present results are also in qualitative agreement with those of our previous investigation in which tretinoin showed higher fluxes through SDS- and ethanol-treated rat skin incomposition to isotretinoin (8).

As mentioned earlier, solubility of these isomers in the donor phase is different. On the other hand, flux is concentration dependent. Therefore, to assess the importance of variables other than concentration in the observed differences, permeability coefficients (Kp) and permeation lag-times of these isomers are also compared. As shown in Table 1, Kp of isotretinoin through oleic acid-treated skin is significantly (P=0.0002) higher than that of tretinoin, a trend and relationship which is opposite to that of flux. This shows that beside concentration, other variables like skin/vehicle partition coefficients and/or diffusion coefficients of drugs through the membrane

Table 1. Permeation of tretinoin and isotretinoin through oleic acid-treated rat skin.

	Flux ($\mu g\ cm^{-2}\ h^{-1}$)	Kp ($cm\ h^{-1}$)	Lag-time (h)
Tretinoin	2.02 ± 0.06	61.23 ± 1.77	11.78 ± 0.67
Isotretinoin	1.61 ± 0.03	70.07 ± 1.36	12.56 ± 0.13
Ratio[a]	1.25	0.87	0.94
P-value[b]	< 0.0001	0.0002	0.062

[a] Tretinoin/isotretinoin
[b] Two-tailed t-test analysis

also play important roles in the observed flux differences.

Permeation lag-time of tretinoin is slightly lower than that of isotretinoin (Table 1), but the difference is not that significant (P=0.062). This might show that diffusion coefficients of retinoids might play a minor role in the permeation rate differences of retinoids. Therefore, the main reason for Kp differences should be partitioning of these drugs into the skin.

These data are partly different from those of our previous study on hydrophilic enhancers (8). Our previous data showed that in SDS-treated skin samples, flux and Kp of tretinoin are significantly higher than those of isotretinoin. In ethanol-treated samples, tretinoin showed higher fluxes than that of isotretinoin at all used ethanol concentrations (25-96%). In terms of Kp, however, the results are different. There was no difference between Kp of retinoids at low ethanol concentrations. But, at higher concentration (96%), Kp of isotretinoin was significantly higher (8), which is in agreement with the present oleic acid data.

SDS affects mainly the intracellaur pathway while oleic acid affects the intercellular lipids, thus opposite permeation trends for retinoids. The mode of action of ethanol depends on its concentration (14) and as shown above, at low concentrations, ethanol tends to act like SDS and at higher concentrations is similar to oleic acid. Our preliminary studies on volatile oils (unpublished data), other well known lipophilic enhancers which mainly affect the intercellular pathway, are in full agreement with the above mentioned results obtained for oleic acid.

These data clearly show that permeation of these retinoids through enhancer-treated rat skin is stereoselective and this phenomenon depends on the polarity and mode of action of enhancers.

Conclusion

Present data show that permeation of tretinoin and isotretinoin through oleic acid-treated rat skin is stereoselective and that the differences between permeability of these retinoids are mainly due to their partitioning into the barrier. Comparison of the present data with those of our previously reported study indicates that the differences between permeability of these retinoids through enhancer-treated skin depend on enhancers' type and their mode of action and possibly the polarity of the permeation pathway.

Therefore, in selection of an isomer or enhancer for transdermal delivery of isomeric drugs, this phenomenon (stereoselectivity) should always be considered. The possibility of isomerisation during storage and after application and its effect on transdermal delivery of such isomers should also be taken into account.

Further studies including the effects of other non-polar enhancers, including volatile oils, on stereoselective permeation of tretinoin and isotretinoin through rat skin are in progress in our laboratories.

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