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Original Article

Stereoselective Permeation of Tretinoin and Isotretinoin through Enhancer-Treated Rat Skin. I. Effect of Ethanol and Sodium Dodecyl Sulfate

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Abstract

Many properties of chemicals depend on their streochemistry. Among these, the effects of streoisomerism on percutaneous absorption of drugs, which is subject of the present investigation, is not well studied yet.

In this study, tretinoin (TT) and isotretinoin (ITT) (geometric isomers) were chosen and their permeations (alone or in the presence of each other) through enhancer-treated excised rat skin were studied. These studies employed static diffusion cells, saturated solution of drugs in water:propylene glycol system as the donor phase and aqueous solution of $Tween_{20}$ as the receptor phase. Aqueous solutions of sodium dodecyl sulfate (SDS) (2, 4, 6 and 8%, w/v) and ethanol (EtOH) (25, 50, 75 and 96%, v/v) were used as enhancers.

Results showed that TT permeates SDS-treated skin by about 1.6-1.8 times faster than ITT ($P<0.0001$). Permeability coefficient (Kp) of TT through SDS-treated skin was also 1.1-1.3 times more than that of ITT ($P<0.004$). In ethanol-treated systems, while the flux of TT was significantly ($P<0.0003$) more than that of ITT (by about 1.4-1.7 times) for all ethanol concentrations, there was no difference between Kp of TT and ITT in 25 and 50% ethanol-treated systems. At higher ethanol concentrations, Kp of TT was significantly ($P<0.046$) more than that of ITT. When the retinoids were used together, flux ratios (TT/ITT) were almost twice of those observed in single-retinoid application studies. These data show that the isomers might affect permeation of each other, which might be due to competition of isomers for permeation through the skin.

The present results clearly show that permeation of these isomers through enhancer-treated rat skin is stereoselective, and that the level of stereoselectivity depends on enhancer type and concentration.

Keywords: Percutaneous absorption; Tretinoin; Isotretinoin; Enhancers; Ethanol; Sodium dodecyl sulfate.

Introduction

Stereoselectivity has been frequently reported in pharmacokinetic, pharmacodynamic and toxicological studies with isomeric drugs, e.g. in drug-receptor interactions and drug metabolism. Permeation of drugs through biological membranes can also be stereoselective. It has been shown that absorption 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).

There are also a few reports regarding stereoselectivity in percutaneous absorption of drugs. It has been shown that percutaneous absorption of diastromers of isosorbide dinitrate is stereoselective (2). It has also been shown that tretinoin permeates human skin faster than its geometric isomer, isotretinoin (3). Stereoselective percutaneous absorption of drugs does not always depend on

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stereoselective permeation through the barrier. It is sometimes due to enzymatic degradation in the skin as is shown for propranolol prodrugs (4).

The success of a transdermal formulation depends on the ability of drug to penetrate the skin barrier at a sufficient rate. Unfortunately, many drugs do not permeate 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 influence the properties of the permeation pathway (7) and even can change the permeation pathway, for example from intercellular to transcellular, as is shown in stratum corneum models (8). Therefore, stereoselective permeation of drugs through normal skin cannot be easily extrapolated to enhancer-treated membrane. Beside this, as the enhancer-membrane interaction depends on enhancer type or concentration (7), such variables can also affect stereoselective permeation of drugs through the skin.

To the best of our knowledge, there is no published data on stereoselective permeation of drugs through enhancer-treated skin, which is the subject of the present investigation. Tretinoin (all-trans retinoic acid, Figure 1) and isotretinoin (13-cis retinoic acid, Figure 1), which are geometric isomers, were chosen as model drugs for this study. Both drugs are available as topical formulations and are used in acne (9).

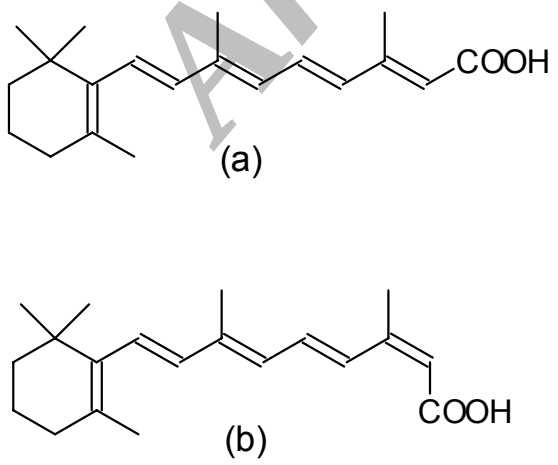

Figure 1. Chemical structures of tretinoin (a) and isotretinoin (b).

Experimental

Material

Tretinoin and isotretinoin (reference standard) were supplied by Roche (Switzerland) and Sigma (USA) respectively. $Tween_{20}$, sodium dodecyl sulfate (SDS) (99%), methanol (99%) and propylene glycol (99%) were purchased from Merck (Germany). Ethanol (96%) was supplied by Bidestan (Iran). 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. The abdominal hairs were then cut by an electrical hair clipper and the 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 the 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 enhancer solution or a control solvent. The system was then stored at room temperature for 12 hr 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 washed twice and then 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 hrs 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 the permeability coefficient was calculated using Fick's Law (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 $Tween_{20}$ 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 (11). Solubility of retinoids in this receptor phase was measured to be around 45 µg/ml.

Saturated solution of drugs in propylene glycol:water (75:25, v/v) system 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. Most experiments employed a donor phase that contained either tretinoin or isotretinoin. As explained earlier, these retinoids are photosensitive and can be degraded to other isomers (3). To study the influence of these isomers on permeation of each other, a preliminary experiment with a donor phase containing both isomers at saturated concentration was performed as well.

Permeation enhancers

For the first part of this study, we decided to use polar enhancers of ethanol and SDS. As the effects of enhancers are usually concentration dependent, we decided to use different concentrations of them. SDS was used as 2, 4, 6 and 8% (w/v) aqueous solutions and ethanol as 25, 50, 75 and 96% (v/v) aqueous solutions. As described earlier, skin samples were pretreated with enhancer solutions prior to application of drugs.

In spite of this method which provides minimal contact between drugs and enhancers throughout the course of the experiment, the effects of enhancers on isomerisation of drugs were also studied. For each enhancer, a 2 ml aqueous solution of enhancer (100 mg/ml) was first added to 2 ml of a 6 mg/ml tretinoin solution in propylene glycol:water (75:25, v/v). The containers were then covered with aluminum foil and stored at room temperature. Retinoid content of the containers was measured by HPLC at the start and after 18 hr. Results were then compared with similar systems containing either propylene glycol:water (75:25, v/v) or water instead of enhancer solutions as the controls. Results showed that there is no difference between isomer contents of enhancer-containing systems and those of controls. During this 18 hr treatment, about 1.5% of tretinoin seemed to have isomerised to isotretinoin, however, the difference was not statistically significant.

Drug measurement

Drug determination was by HPLC and UV spectrophotometric assay. HPLC method was used for those experiments in which it was necessary to measure the retinoids separately. These experiments included effects of enhancers on isomerisation of retinoids, co-administration of retinoids in permeation studies and finally confirmation of accuracy of the UV method.

UV spectrophotometry

UV spectrophotometric method was performed at 360 nm using a Spectronic 601 spectrophotometer (Milton-Roy, USA). Standard working curves were constructed from known concentrations of drugs in the solutions. To evaluate the possibility of release of chemicals from skin and interaction of these materials with the UV drug assay method, different control permeation studies were performed. These studies used propylene glycol: water solution (75:25, v/v, without any drug) as the donor phase and performed on both

ethanol and SDS pretreated skin samples. Results showed that there is no interference with the measurement from skin material. The accuracy of the UV method in measurement of both drugs was confirmed by the HPLC method.

High Performance Liquid chromatography

A HPLC method developed by Zarghi and co-workers (12) was employed here for measurement of tretinoin and isotretinoin. Perkin-Elmer HPLC system with 501 dual piston pump, C_{18} column and U6K injector was used. UV absorbance of the elute was monitored at 360 nm on a 486 variable wavelength spectrophotometer. Methanol:water (80:20, v/v) at a flow rate of 1.3 ml/min was used as the mobile phase. Standard solutions of tretinoin and isotretinoin (20 μg/ml) were freshly prepared and daily basis, and their corresponding peak height was used to measure retinoid concentrations in samples.

Results and Discussion

Permeation of retinoids through untreated skin

Permeation of tretinoin and isotretinoin in the absence of enhancers through full-thickness rat skin was rather low and we were not able to detect the retinoids in the receptor phase even by HPLC method. 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 $ng.cm^{-2}.hr^{-1}$ through human skin at maximum thermodynamic activities of tretinoin and isotretinoin. They also measured the permeation flux of isotretinoin through monkey skin to be around 1 $ng.cm^{-2}.hr^{-1}$ (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 our HPLC method (10 ng/ml).

Permeation of retinoids through SDS-treated skin

Figure 2 shows the flux of tretinoin and isotretinoin through 2-8 % SDS-treated rat skin. These results show that at a saturated concentration (maximum thermodynamic activity), tretinoin permeates SDS-treated rat skin 1.6-1.8 times faster than isotretinoin

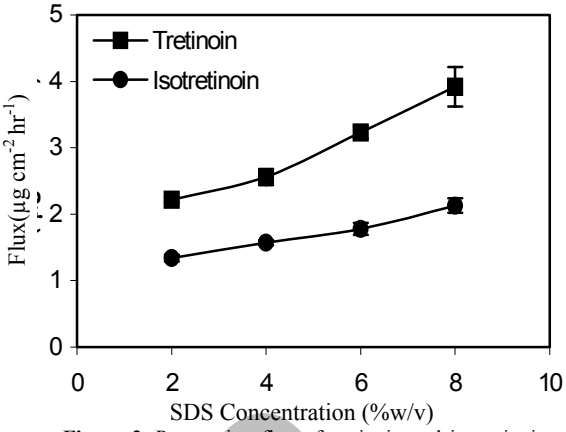

Figure 2. Permeation flux of tretinoin and isotretinoin through sodium dodecyl sulfate (SDS)-treated rat skin at room temperature. Data are Mean ± SD, n = 4.

(P<0.0001). These results are in agreement with those reported by Lehman et al. (3) for human skin. They applied these retinoids as isopropyl alcohol solution and evaporated the solvent after application to the skin. In such a condition, during evaporation, thermodynamic activity of drug increases until saturation reaches, at which concentration maximum flux is expected. Therefore, in terms of thermodynamic activity, our system and that of Lehman and co-workers should be the same. Their results show that permeation flux (as measured based on drug concentration in the receptor phase) of tretinoin through untreated human skin is almost twice that of isotretinoin (3).

As mentioned earlier, solubility of these isomers in the donor phase are different. On the other hand, flux is concentration dependent. Therefore, to access the importance of variables other than concentration in the differences

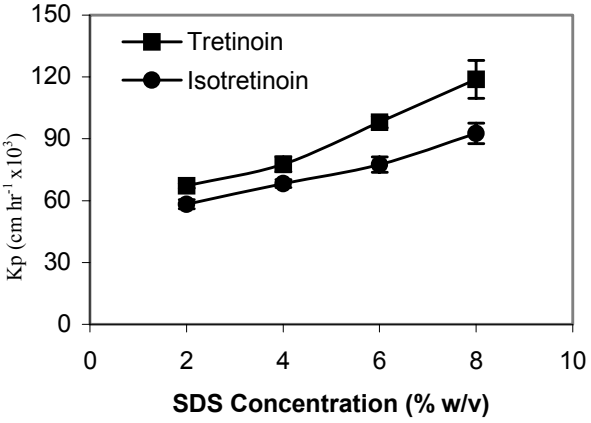

Figure 3. Permeability coefficient of tretinoin and isotretinoin through sodium dodecyl sulfate (SDS)-treated rat skin at room temperature. Data are Mean ± SD, n = 4.

between tretinoin and isotretinoin fluxes, we compared the permeability coefficient (Kp) of these isomers as well. Figure 3 shows permeability coefficient of tretinoin and isotretinoin through SDS-treated rat skin. The Kp of tretinoin was found to be around 1.1-1.3 times greater than that of isotretinoin, which is also statistically significant (P<0.004). This shows that beside concentration, other variables like skin/vehicle partition coefficients and diffusion coefficients of drugs through the skin also play role in the differences observed between drugs' permeation rates through SDS-treated rat skin. These data clearly show that permeation of these retinoids through SDS-treated rat skin is stereoselective.

Figures 2 and 3 show that flux and permeability coefficient of both isomers through SDS-treated rat skin increase as a result of an increase in the SDS concentration.

Permeation of retinoids through ethanol-treated skin

Figure 4 shows the flux of tretinoin and isotretinoin through 25-96% ethanol-treated skin. These results show that at a saturated concentration (maximum thermodynamic activity), permeation flux of tretinoin through ethanol-treated rat skin is around 1.4-1.7 times more than that of isotretinoin (P<0.0003). These results are qualitatively in agreement with those reported for permeation of these retinoids through untreated human skin (3) and those observed in our SDS-treated rat skin described above.

Figure 5 shows the permeability coefficient (Kp) of tretinoin and isotretinoin through ethanol-treated rat skin. Our results show that although the flux of isotretinoin in 25% ethanol–treated skin is significantly lower than that of tretinoin, its Kp is slightly higher, although, the difference is not significant (P = 0.584). When the ethanol concentration increases up to 50%, the ratio reverses and the Kp of isotretinoin becomes lower than that of tretinoin, but the difference is still not significant (P = 0.187). At higher ethanol concentrations (75 and 96%), the difference between Kp of tretinoin and isotretinoin becomes more and also statistically significant (P<0.046).

These data show that at low ethanol concentrations, there is no difference between Kp of retinoids and the difference between fluxes can be explained by difference in concentrations. These data are not in complete agreement with those observed in SDS-treated skin. As the concentration of ethanol increases, variables other than concentration also become important and play significant roles in flux differences. Our results also show that the Kp ratio (tretinoin/isotretinoin) depends on ethanol concentration. This is mainly due to higher enhancement ratio of tretinoin than that of isotretinoin, when the ethanol concentration is increased from 25% to 96%, as can be seen by comparing the slope of graphs in Figure 5.

The data presented in this study show that not only permeation of tretinoin and isotretinoin through enhancer-treated rat skin is stereoselective, but also their response to enhancers are different and could be considered stereoselective. This response is concentration dependent and in addition also depends on the enhancer type.

Co-administration of retinoids

In a preliminary study, we applied both drugs to skin together. This study was

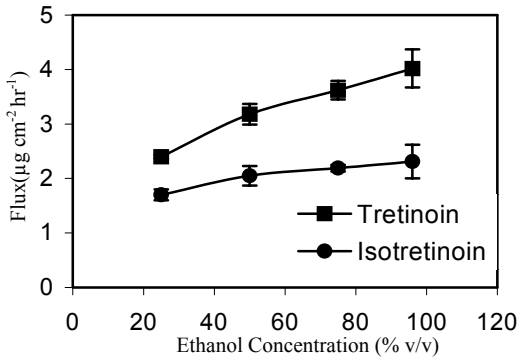

Figure 4. Permeation flux of tretinoin and isotretinoin through ethanol-treated rat skin at room temperature. Data are Mean±SD, n=4.

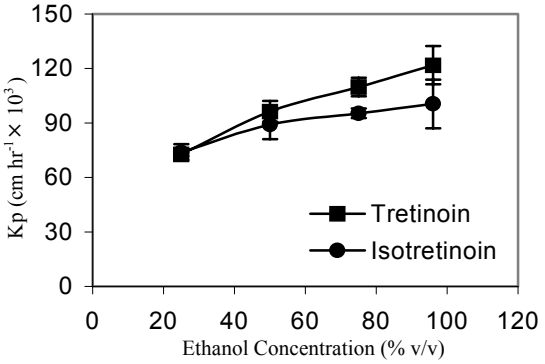

Figure 5. Permeability coefficient of tretinoin and isotretinoin through ethanol-treated rat skin at room temperature. Data are Mean ± SD, n = 4.

Table 1. Permeation of tretinoin and isotretinoin through enhancer-treated rat skin when applied together.

	Flux (□g cm^{-2} hr^{-1})			
Enhancer	Tretinoin	Isotretinoin	Flux ratio	P value*
SDS	4.46 ± 0.94	1.23 ± 0.38	3.63	0.0007
Ethanol	4.96 ± 1.39	1.44 ± 0.30	3.44	0.0026

* Two-tailed t-test analysis

performed on both 8% SDS-treated and 96% ethanol-treated skin samples. Figure 6 shows sample peaks of tretinoin and isotretinoin as detected in the receptor phase in enhancer-treated skin. Results (Table 1) show that in such a condition, tretinoin permeates skin 3.5 times faster than isotretinoin. Flux ratios (tretinoin/isotretinoin), when applied separately (Figures 2 and 4), were 1.84 and 1.74 in 8% SDS- and 96% ethanol-treated systems respectively, which are nearly half the ratio observed in co-administration.

These data show that the increased flux ratio observed is due to both an increase in tretinoin flux, as well as a decrease in isotretinoin flux. This could not be due to changes in solubility of drugs, for example due to salting out of isotretinoin by tretinoin. Because the system is still saturated with both drugs, in such a circumstance maximum fluxes for both drugs are expected. We did not measure the solubility of drugs in the donor phase under this condition, in which the system was saturated with both drugs and amounts of drug excess powders present in the system.

Another possibility would be the enhancement effects of retinoids. It has been shown that tretinoin increases percutaneous absorption of minoxidil (13). Isotretinoin is in *cis* conformation (Figure 1) and molecules in such a conformation are expected to show greater enhancement effects than that of their *trans* isomers (14). So, isotretinoin might have acted as an enhancer for tretinoin or even itself. Comparison of the slopes of flux vs. concentration graphs in both SDS and ethanol-treated systems (Figures 2 and 4) show that the slope of tretinoin system is more than that of the isotretinoin system. This might indicate the higher sensitivity of tretinoin to enhancement than that of isotretinoin and show that any further increase in fluxes increases the gap between tretinoin and isotretinoin; a point that needs further investigation. However, this explanation cannot explain the decrease observed in isotretinoin flux.

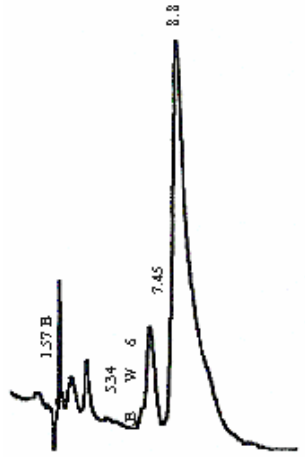

Figure 6. Sample HPLC chromatogram from the receptor phase showing the permeated isotretinoin (retention time = 7.45 min) and tretinoin (retention time = 8.80 min) through 8% SDS-treated rat skin.

Transformation of isotretinoin to tretinoin would be another possibility. However, it has been shown that when the system is kept in dark, isomerisation of isotretinoin would be less that 1%, and more than 90% of isotretinoin would stay intact (3). Our results show that the increase in tretinoin flux is about 15-20%, and the decrease in isotretinoin flux is about 60-70%.

Other possibilities like competition of isomers for permeation through the skin or binding to skin components also exists and could play an important role in the observed differences. All of the above mentioned possibilities require further investigation; which are in progress in our laboratories.

Present data show that permeation of tretinoin and isotretinoin, and possibly other isomers, through enhancer-treated rat skin is stereoselective. Beside this, the response of isomers to enhancers is also different and can be considered stereoselective. Our investigations also reveal that the stereoselectivity might be concentration-dependent as well. Therefore, in selection of an isomer for transdermal delivery, this phenomenon (stereoselectivity) should be always kept in mind and be considered. The possibility of isomerisation during storage and after application to skin and its effect on transdermal delivery of such isomers should also be taken into account.

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

Acknowledgments

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