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Monohydroxylation and esterification as determinants of the effects of *cis*- and *trans*-9-octadecenoic acids on the permeation of hydrocortisone and 5-fluorouracil across hairless mouse skin in vitro

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Abstract

The effects of *cis*-9-octadecenoic acid (oleic acid) and of a group of chemically related *cis*- (ricinoleic acid) and *trans*- (ricinelaidic acid) 12-monohydroxylated derivatives and their corresponding ethyl and methyl esters on the skin permeation of model hydrophobic (hydrocortisone, log $K = 1.61$) and hydrophilic (5-fluorouracil, log $K = -0.89$) drugs was investigated in vitro using excised hairless mouse skin. Drug solutions were prepared in propylene glycol, with and without the addition of a fatty acid to a level of 5%. Whereas the addition of oleic acid markedly enhanced the transdermal flux of both drugs relative to a sample in propylene glycol alone (hydrocortisone ~ 1800-fold; 5-fluorouracil ~ 330-fold), that of a *cis*- or *trans*-12-monohydroxylated analog of oleic acid resulted in only a small increase (1.4–2.7-fold for hydrocortisone; 4.4–6.6-fold for 5-fluorouracil). On the other hand, the methyl and ethyl esters of *cis*- and *trans*-12-hydroxy-9-octadecenoic acid exerted a much greater enhancing effect (327–720-fold for hydrocortisone, 42–74-fold for 5-fluorouracil) than the corresponding parent fatty acids. Furthermore, whereas the ethyl esters were found to have a greater effect on the skin permeation of hydrocortisone than the methyl esters, the reverse was true with regards to 5-fluorouracil. Additionally, the esters of *trans*-12-hydroxy-9-octadecenoic acid promoted permeation to an extent comparable to that achieved with their *cis*-counterparts.

Keywords: Cis- and trans- 9-octadecenoic acids; Monohydroxylation; Esterification; Hydrocortisone; 5-Fluorouracil; Skin permeation

1. Introduction

Owing to its anatomical structure, biochemical composition and molecular organization, the stratum corneum, the outermost layer of the skin, is generally regarded as the primary barrier to the

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penetration of drugs following their topical application (Scheuplein and Blank, 1971). This barrier can be made more permeable to penetrant solutes by including skin permeation enhancers in the topical drug formulation (Barker and Hadgraft, 1981; Cooper et al., 1985; Sarpotdar and Zatz, 1986; Barry and Bennett, 1987; Sato et al., 1988; Mahjour et al., 1989; Schneider et al., 1996). Anionic adjuvants in the form of various fatty acids have been shown to increase transdermal delivery of drugs with a wide range of polarities (Cooper, 1984; Cooper et al., 1985; Green and Hadgraft, 1987; Green et al., 1988; Aungst et al., 1990; Morimoto et al., 1995, 1996). For the fatty acids thus far examined, the accumulated evidence has indicated that those with olefinic bonds are generally more effective as skin penetration enhancers than those without (Cooper, 1984; Aungst et al., 1986; Loftsson et al., 1995), and that *cis*-monoolefinic compounds with carbon chains longer than ten-carbons and the unsaturation centrally located are more effective than the corresponding homologous *trans*-isomers (Golden et al., 1987a). In this regard, for example, oleic acid is reported to be between 100–300-fold more effective than the C_{14}–C_{18} saturated fatty acids, myristic, palmitic or stearic acids, in enhancing the flux of hydrocortisone in a propylene glycol vehicle through hairless mouse skin (HMS), and that such an enhancing effect is directly proportional to the amount of oleic acid present (Loftsson et al., 1995).

An early view to account for the increased rate of transport of lipophilic drugs across the skin has been that an unsaturated fatty acid can make the stratum corneum more permeable to the coapplied drug by interacting with the lipid arrays of this skin structure so as to increase the motional freedom or flexibility of their hydrocarbon chains (the so-called disordering effect) and, hence, more permeable to solute flux (Mak et al., 1990a,b) A more recent and accepted concept, based on IR spectroscopic, calorimetric and flux techniques (Golden et al., 1987a,b; Barry and Bennett, 1987; Francoeur et al., 1990; Potts et al., 1991; Walker and Hadgraft, 1991) is that an unsaturated fatty acid such as oleic acid, plus or minus a polar solvent such as propylene glycol, will lower the diffusional resistance of the stratum corneum to polar and relatively polar solutes by lowering the thermal phase properties of the lipid components without affecting that of keratin. In this context, since at physiological temperatures oleic acid is found to exist as a liquid within the lipid bilayers of the stratum corneum, it will bring about the formation of permeable interfacial defects within this skin layer that will effectively reduce the diffusional path length or resistance (the fluid–solid phase separation effect) without necessarily promoting the formation of pores (Ongpipattanakul et al., 1991).

The aim of the present study was to evaluate the combined effect of 12-hydroxylation, esterification and *cis–trans* geometrical isomerism across the double bond of 9-octadecenoic acid on transdermal drug permeation. These interactions have been examined in vitro using excised HMS, model hydrophobic (hydrocortisone) and hydrophilic (5-fluorouracil) drugs, oleic acid and several free and esterified *cis*- and *trans*-12-hydroxy-9-octadecenoic acids. To our knowledge, this subject has not been previously investigated.

2. Materials and methods

2.1. Materials

Hydrocortisone (HC) and 5-fluorouracil (5FU) were obtained from Aldrich Chemical Company (Milwaukee, WI) and used as received. Unsaturated fatty acids (oleic acid = OA, ricinoleic acid = RCA, ricinelaidic acid = RDA), fatty acid esters (ethyl ricinoleate = RCE, methyl ricinoleate = RCM, ethyl ricinelaideate = RDE, methyl ricinelaideate = RDM), propylene glycol (PG), polyethylene glycol (PEG) 400, sodium chloride, glacial acetic and formaldehyde were purchased from Sigma Chemical Company (St. Louis, MO). The solvents for the liquid chromatographic analyses were of HPLC grade and from E.M. Science, Gibbstown, NJ.

2.2. Solubility studies

The solubilities of both HC and 5FU were investigated by adding an excess amount of drug to 1 ml of PG placed in a screw-capped glass vial, in the absence or presence (5% w/v) of a fatty acid or fatty acid ester. After tightly closing the vials, their contents were mechanically shaken at 25°C for 24 h. The suspensions were then centrifuged at 6000 rpm for 15 min, and the supernatant solutions, or suitable dilutions, were assayed for drug using HPLC methods.

2.3. Test solutions for permeation studies

The solutions for transdermal permeation studies were prepared by placing a test compound (HC, 15 mg; 5FU, 5.4 mg), plus or minus an unsaturated fatty acid or fatty acid ester (50 mg each) in a screw-capped glass vial, adding PG to a final volume of 1 ml, and sonicating the mixture to effect complete dissolution.

2.4. Skin permeation studies

Permeation studies were conducted using side-by-side Valia-Chien type diffusion cells (Crown Glass Company, Somerville, NJ), and full-thickness abdominal skin that had been surgically removed with the aid of a pair of forceps from female hairless HRS/J mice (6–8 weeks old, Jackson Laboratories, Bar Harbor, ME). The skin section was mounted on the receptor phase compartment of the diffusion apparatus (available diffusion area of 0.64 cm^2), with the stratum corneum facing the donor phase. The drug solution (3.5 ml, containing 15 mg/ml of HC or 5.4 mg/ml of 5FU) was placed in the donor compartment. The receptor compartment was filled with an equal volume of 30% PEG 400 in normal saline (HC experiments) or of normal saline containing 0.1% formaldehyde (5FU experiments). The temperature of the cells and their contents was kept at a constant value of 32 ± 0.5°C throughout the experiment while stirring the receptor phase magnetically with a Teflon-coated magnetic bar rotating at 500 rev/min. In all cases, 100 μl of receptor phase was removed with a microsyringe at 1, 2, 3, 4, 5, 6, 8, 12, 24, 36 and 48 h, and replaced with an equal volume of fresh receptor phase. The samples were kept in the freezer until analyzed for drug content using an HPLC method. All permeation studies were conducted in triplicate. Cumulative corrections were made to determine the total amount of drug that had permeated at each time interval. The steady state flux of the drug, J_{ss}, in μg/cm^2/h, was calculated using the following equation:

$$J_{ss} = 1/A(\mathrm{d}M/\mathrm{d}t) = P\Delta C$$

where A is the diffusional area of the skin section in cm^2, $\mathrm{d}M/\mathrm{d}t$ is the slope of the linear portion of the permeation curve in μg/h, P is the apparent permeability coefficient in cm/h, and ΔC is the concentration gradient in μg/ml. The value of lag time was obtained by extrapolating the linear portion of the steady-state permeation curve to the time axis.

2.5. Drug assays

The concentrations of HC and 5FU in the various samples were determined by reversed-phase HPLC methods with spectrophotometric detection. The liquid chromatograph consisted of Model 250 binary pump (Perkin–Elmer Corporation, Norwalk, CT), Spectroflow 783 absorbance detector (Kratos Analytical, Ramsey, NJ), Model AS 4000 autoinjector (Hitachi Instruments, Inc., San Jose, CA), and Model 3396 integrator (Hewlett-Packard, Avondale, PA). The analysis of HC was conducted on a Supelcosil LC-18, 5 μm, 150×4.5 mm, column (Supelco, Bellefonte, PA) using methanol–water–acetic acid (43:52:5) as the mobile phase, and a flow rate of 1.5 ml/min. At a detection wavelength of 241 nm, HC eluted at about 4.1 min. 5FU was analyzed on a μBondapak C_{18}, 10 μm, 300×3.9 mm, column (Waters Corporation, Milford, MA), with water as the mobile phase, and a flow rate of 1 ml/min. At a wavelength of 266 nm, 5FU eluted at about 7.0 min. Following appropriate dilutions, the sample preparations were injected into the liquid chromatograph in a volume of 10–50 μl. Drug concentrations were calculated on the basis of peak areas and with reference to calibration

curves prepared from a series of standard solutions of HC or 5FU covering the concentration ranges 1–500 μg/ml. The HPLC methods were able to accurately measure as little as 30 ng/ml of HC and 100 ng/ml of 5FU.

2.6. Data analysis

Experimental results are reported as the mean ± SD for triplicate experiments. Statistical comparisons were made by the Student *t*-test, and the values were considered to be significantly different at $p < 0.05$.

3. Results and discussion

Table 1 shows the effect of three monounsaturated fatty acids, i.e., OA (*cis*-9-octadecenoic acid), RCA (*cis*-12-hydroxy-9-octadecenoic acid) and RDA (*trans*-12-hydroxy-9-octadecenoic acid), on the solubility and permeation parameters through excised HMS of HC, a relatively lipophilic drug (log $K = 1.61$). For test formulations containing concentrations of HC in PG representing 72–85% of saturation, the addition of a monounsaturated fatty acid (5%) resulted in only a slight increase (10–13%) in drug solubility. On this basis, it was assumed that the thermodynamic driving forces among the various test formulations would not be sufficiently different from one another to account for significant differences in their permeation characteristics. However, as inferred from the data shown in Fig. 1 and Table 1, this was not the case. Thus, whereas the transdermal permeation rate of HC from PG yielded a long lag time (6.4 h) and was very slow, in the presence of OA it became accelerated as well as enhanced ~ 1800-fold. In contrast, the introduction of a 12-hydroxyl group into the carbon chain of 9-octadecenoic (like in RCA and RDA) resulted in a marked attenuation ($p < 0.001$) of the enhancing effect of OA (increases of < 1.5-fold with RCA and < 3-fold with RDA). In the case of the two 12-hydroxy-lated analogs of OA tested, the *trans*-isomer RDA was found to exert an insignificantly greater enhancing than the corresponding *cis*-isomer RCA (2.7-fold vs 1.4- fold).

Table 1
Skin permeation parameters of hydrocortisone across the hairless mouse skin from propylene glycol containing 5% oleic acid, *cis*- and *trans*-12-hydroxyoleic acids, and their esters.

Vehicle	C_s[a] (mg/ml)	T_{lag}[b] (h)	J_{SS}[b] (μg/cm²/h)	EF[c] for HC
PG	17.6	6.35 ± 2.19	0.10 ± 0.02	1.00
5%OA-PG	19.6	1.70 ± 0.15	98.12 ± 6.06	981.20
5% RCA-PG	19.9	2.76 ± 2.47	0.14 ± 0.04	1.40
5%RCM-PG	20.1	3.26 ± 0.16	2.20 ± 0.24	22.00
5%RCE-PG	20.3	3.63 ± 0.14	9.12 ± 1.50	91.20
5%RDA-PG	19.3	< 0.5	0.27 ± 0.10	2.70
5% RDM-PG	20.8	3.49 ± 0.02	5.64 ± 0.75	56.40
5%RDE-PG	20.8	3.42 ± 0.09	10.16 ± 0.57	101.60

[a] Solubilities were determined at 25°C.
[b] Steady-state fluxes and lag times were determined using a 15 mg/ml drug solution; Values are presented as mean ± S.D. ($n = 3$).
[c] Enhancement factor = $J_{SS,\ vehicle}/J_{SS,\ PG}$.

In the present study, differences in cutaneous penetrating enhancing effects for HC were noted between *cis*- and *trans*-12-hydroxylated 9-octadecenoic acids. However, in contrast with the results of earlier studies on indomethacin (Morimoto et al., 1996) and salicylic acid (Golden et al., 1987b), monohydroxylation of the 9-octadecenoic acid molecule appeared to either attenuate or negate the greater disturbing effect among the lipid domains of the stratum corneum that would be expected from a symmetrically located natural 'kink' of 120° of a *cis*-isomer of 9-octadecenoic

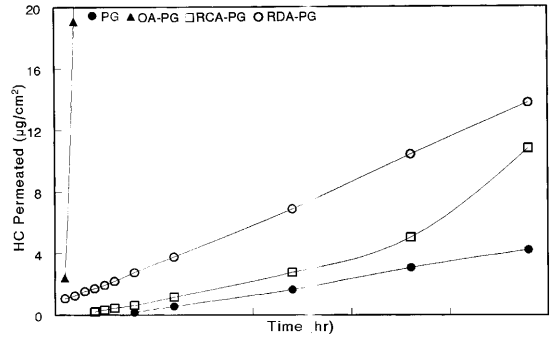

Fig. 1. Skin permeation profiles of HC across HLM skin at 32°C from 5% solutions of *cis*- and *trans*-hydroxylated and nonhydroxylated 9-octadecenoic acids in PG. Each point is the mean of three experiments.

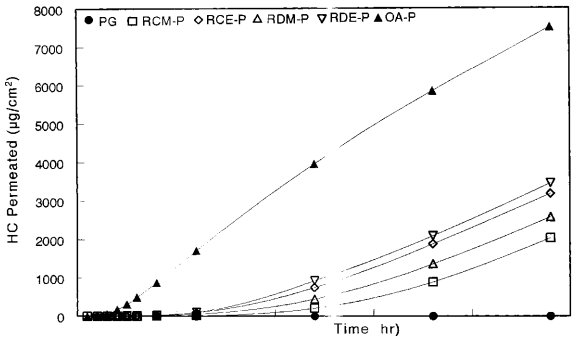

Fig. 2. Skin permeation profiles of HC across HLM skin at 32°C from 5% solutions of *cis*- and *trans*-12-hydroxy-9-octadecenoic acid esters in PG. Each point is the mean of three experiments.

acid than from the more 'straight' homologous *trans*-isomer (Walker and Hadgraft, 1991).

From the data presented in Table 1 and Figs. 1 and 2, it is apparent that the esterification of RCA and RDA will have a significant positive effect on the permeation of HC through HMS in comparison to the corresponding parent fatty acids. Thus, relative to RCA, drug flux in the presence of its methyl and ethyl esters was about 543-fold and 720-fold higher, respectively ($p < 0.001$ in both cases). A similar trend was observed between RDA and its methyl and ethyl esters, with the enhancement being about 327-fold higher with the methyl ester and 388-fold higher with the ethyl ester ($p < 0.001$ for both).

The general trend of the esterified 12-hydroxylated-9-octadedenoic acids to exert a greater enhancing effect on the transdermal permeation of HC across HMS than their corresponding parent fatty acids could be the results of their greater hydrophobicity and viscosity. These factors in turn could contribute to their more pronounced altering effect on nonpolar, esterified fatty acid-rich, pathways within the stratum corneum that contribute to diffusional resistance (Golden et al., 1987b)

As indicated in Table 2 and Fig. 3 the addition of OA, RCA and RDA, and of a methyl or ethyl ester of RCA or RDA to a solution of 5FU in PG resulted in a small reduction (3–7%) in drug solubility. For formulations containing 5FU in PG in concentrations representing 31–33% of saturation, the addition of the same adjuvants produced transdermal permeation results that followed trends, similar to those noted with HC despite being different in magnitude. Thus, fluxes were again much higher in the presence of OA (333-fold increase, $p < 0.001$) than in the presence of a *cis*- (< 5-fold increase) or *trans*- (< 7-fold increase) 12-hydroxylated analog. Also, as noted with HC, the methyl and ethyl esters of the *cis*- and *trans*-12-hydroxylated analogs of OA were able to enhance transdermal permeation to a much greater extent (42-74-fold higher, $p < 0.001$) than the corresponding unesterified fatty acids (Table 2 and Fig. 4).

Table 2
Skin permeation parameters of 5-fluorouracil across the hairless mouse skin from propylene glycol containing 5% oleic acid, *cis*- and *trans*- 12-hydroxyoleic acids, and their esters.

Vehicle	C_s[a] (mg/ml)	T_{lag}[b] (h)	J_{SS}[b] (μg/cm²/h)	EF[c] for FU
PG	17.4	1.84 ± 1.34	0.30 ± 0.06	1.00
5% OA-PG	16.4	0.58 ± 0.29	121.69 ± 4.90	405.63
5% RCA-PG	16.6	3.30 ± 2.47	1.02 ± 0.19	3.40
5% RCM-PG	16.8	3.63 ± 0.43	28.12 ± 8.36	93.73
5% RCE-PG	16.2	2.67 ± 0.09	23.38 ± 4.35	77.93
5% RDA-PG	16.2	1.37 ± 0.82	1.32 ± 0.89	4.56
5% RDM-PG	16.6	3.53 ± 0.35	53.18 ± 8.67	145.77
5% RDE-PG	16.7	2.24 ± 0.41	29.86 ± 5.97	99.53

[a] Solubilities were determined at 25°C.
[b] Steady-state fluxes and lag times were determined using a 5.4 mg/ml drug solution; Values are presented as mean + S.D. ($n = 3$).
[c] Enhancement factor = $J_{SS,\ vehicle}$ $J_{SS,\ PG}$.

However, in contrast with the results gathered for HC, the methyl esters were more effective in promoting 5FU skin permeation than the ethyl esters (323-fold mean increase vs 238-fold mean increase.

When comparing the transdermal flux values of HC, a relatively hydrophobic drug, with those of 5FU, a hydrophilic drug, it is apparent that, at the concentrations of fatty acid coapplied, the flux of the former was less than that of the latter from the PG vehicle.

Likewise, the addition of a *cis*- or *trans*-monohydroxylated analog of OA to PG exerted a greater enhancing effect on the transdermal permeation of 5FU than on that of HC. In contrast, the inclusion of the relatively hydrophobic OA or of a *cis*- and *trans*-monohydroxylated ester of OA in PG produced a greater promoting effect on the skin permeation of HC than on that of 5FU. These results could reflect differences in routes of permeation for each compound through the stratum corneum as well as differences in the disturbance that each type of fatty acid and its ester can bring about within the lipid domains of this skin layer. Indeed, as suggested by the correlations between skin permeability and partition coefficients made by Barry (1987), distinct hydrophilic (polar) and lipophilic (nonpolar) pathways may exist for hydrophilic and hydrophobic drugs, respectively. Furthermore, experimental verification of this postulate have come from work by Yamashita et al. (1994, 1995) and others (Koyama et al. 1994) who, by analyzing the skin permeation of drugs based on a two-layer model with polar and nonpolar routes in the stratum corneum, have found that the action of a permeation enhancer can be discussed in terms of drug diffusivity and partition coefficient in each domain.

In accordance with this diffusion model, it would appear that a relationship exists between each of these pathways and the actions of different types of OA compounds. Thus, the addition of a relatively hydrophilic monounsaturated-monohydroxylated analog of OA to a drug formulation will have a greater effect on the in vitro skin permeation of a hydrophilic (i.e. 5FU), than on that of a more lipophilic (i.e. HC), substance as a result of a greater increase in drug partition parameter for the nonpolar route. This view is supported by observations that OA can promote transepidermal water loss (Green et al., 1988) at the fluid–solid interfaces of lipids of the stratum corneum associated with water (Francoeur et al., 1990; Ongpipattanakul et al., 1991) to create interfacial defects leading to enlargement of the polar route (Yamashita et al., 1995). In this respect, although the hydroxylated esterified derivatives of RCA and RDA were also found to enhance the transdermal flux of 5FU to a greater extent than that of HC, and more so than the corresponding unesterified parent fatty acids, their diminished effects relative to that of OA might be related to their lesser tendency to deprive the stratum corneum of water.

In conclusion, the present study has found that 12-hydroxyl derivatives of cis- and *trans*-9-octadecenoic acid will enhance the in vitro transdermal permeation of a hydrophilic drug such as 5FU to a greater extent than that of the more hydrophobic HC. However, relative to an OA-containing formulation, the enhancing effect demonstrated by these monohydroxylated unsaturated fatty acids was markedly less. While esterification of these *cis*- and *trans*-hydroxylated derivatives of 9-octadecenoic acid with a low molecular weight

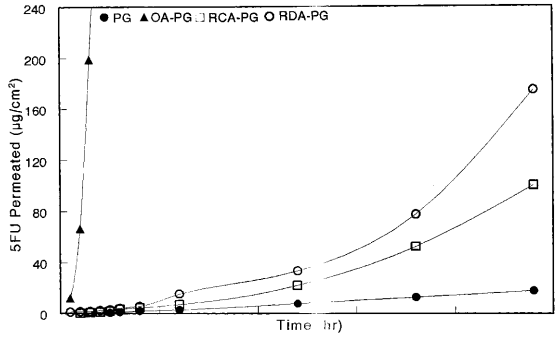

Fig. 3. Skin permeation profiles of 5FU across HLM skin at 32°C from 5% solutions of *cis*- and *trans*-hydroxylated and nonhydroxylated 9-octadecenoic acids in PG. Each point is the mean of three experiments.

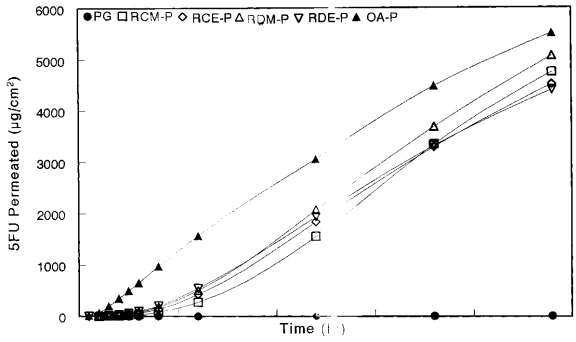

Fig. 4. Skin permeation profiles of 5FU across HLM skin at 32°C from 5% solutions of *cis*- and *trans*-12-hydroxy-9-octadecenoic acid esters in PG. Each point is the mean of three experiments.

alkanol improved the transdermal enhancing effect of the unesterified parent fatty acid, the enhancement was still rather small when compared to that attained in the presence of OA. A future study aimed at verifying whether or not the correlations found here are observable under in vivo conditions appears to be warranted.

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