Synthesis and Effect of Two New Penetration Enhancers on the Transdermal Delivery of 5-Fluorouracil through Excised Rat Skin

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The tetrahydrogeraniol (THG) derivative, ethyl-(3,7-dimethyl octyl thio) acetate (EDOTA) was prepared by reacting tetrahydrogeranyl bromide (obtained by reaction of 40% hydrobromic acid and concentrated sulfuric acid) with ethyl 2-mercaptoacetate, while 3,7-dimethyl octyl propionate (DOP) was synthesized by a common esterification reaction by reacting THG with propionic acid in the presence of cyclohexane and concentrated sulfuric acid. The penetration-enhancing effect of the new enhancers were compared with THG and Azone *in vitro* using excised rat skin in modified Franz-type diffusion cells. 5-Fluorouracil (5-FU), a hydrophilic drug with poor skin permeability was used as a model permeant. Skin samples were pretreated with pure liquid enhancers for 12 h. 5-FU flux through the control and enhancer-treated skin increased linearly with its concentration in the receptor compartment. EDOTA and DOP interacted with the skin rapidly (<2 h), and the duration of action is at least 24 h. Significant differences were found in the flux values of 5-FU; EDOTA and DOP enhanced the permeability of the drug about 6-fold and 11-fold respectively. Increased partition coefficient and diffusion coefficient values were obtained by these enhancers. The results suggested that the amount of EDOTA and DOP in the skin, especially in the stratum corneum, may be related to their penetration-enhancing effect.

Key words tetrahydrogeraniol; 5-fluorouracil; percutaneous absorption; ethyl-(3,7-dimethyl octyl thio) acetate; 3,7-dimethyl octyl propionate; rat skin

The transdermal delivery of drugs for topical treatment or systemic disorder has received much attention. The outer layer of skin, the stratum corneum (SC) is generally recognized as the primary barrier to transdermal delivery of drugs. The SC is thin, heterogeneous structure comprising stacked layers of terminally differentiated and keratinized epidermal cells distributed in a complex, lamellar, intercellular domain. The SC intercellular lipids largely dictate the overall skin permeation properties.[1] Substances which are able to modulate the skin barrier and allow higher amounts of drugs to penetrate the skin are so-called skin permeation enhancers.[2] These agents increase drug transport through the skin by increasing the partition and/or diffusion coefficient. An ideal enhancer should increase drug transport by reversibly altering the skin barrier function without sensitization or irritation.[3] Therefore, the possibility of using many synthetic or naturally occuring compounds as effective enhancers has been investigated. Essential oils in citrus fruits, such as lemon or orange, have been widely used as flavoring substances for perfumes, foodstuffs and medicines. The biological safety of these compounds has been well documented.[4] Recently, we found that geraniol, an acyclic monoterpene present in the oil of rose and ylang-ylang markedly enhanced the percutaneous absorption of 5-fluorouracil (5-FU).[5]

The present study extends the investigation to the synthesis and enhancing effects of ethyl-(3,7-dimethyl octyl thio) acetate (EDOTA) and 3,7-dimethyl octyl propionate (DOP), a tetrahydrogeraniol (THG) derivative, on transdermal permeation of 5-FU through excised rat skin. The permeation enhancement found with EDOTA and DOP was compared with THG (the parent compound) and Azone.

Experimental

Apparatus IR spectra were obtained on a Perkin Elmer 983 IR spectrophotometer. ^{1}H-NMR spectra were recorded on a PMX-60si spectrometer. Mass spectra were obtained with a ZAB-HS-VG analytical organic mass spectrometer. GC was performed with a Shimadzu gas chromatograph GC 9A. TLC studies were made on silica gel plates with ethyl acetate as the solvent and iodine as the visualization agent.

Reagents and Solvents THG was purchased from Nanjing Perfume Factory and was purified to 99.9%. 5-FU and Azone were purchased from Shanghai 12th Pharmaceutical Manufacturing Factory and Guangzhou Zhuji Chemical Factory respectively. All other chemicals and reagents used were of analytical grade obtained commercially.

Preparation of Tetrahydrogeranyl Bromide A 500 ml three-necked flask was equipped with an electrical stirrer, a dropping funnel and a reflux condenser. 40% hydrobromic acid (126 ml) was added to the flask, followed by THG (84.7 g, 0.43 mol) dropwise and concentrated sulfuric acid (24.8 ml, 0.465 mol) and stirred at room temperature for an hour. The reaction mixture was heated to 130 °C and fluxed for 10 h. Then the reaction mixture was cooled and the upper brown-black liquid was washed twice with water, then with 2% sodium bicarbonate solution till the pH value was about 7.0. Then, it was dried with anhydrous magnesium sulfate for 2 h. The residue was distilled on an oil bath at 95 °C. The distillate collected at 58—62 °C gave tetrahydrogeranyl bromide (74.4 g, 78.7%) as an yellowish liquid.

Preparation of Ethyl 2-Mercaptoacetate A mixture of thioglycolic acid (17 g, 0.184 mol), anhydrous ethanol (30 ml, 0.51 mol), cyclohexane (30 ml) and concentrated sulfuric acid (1.3 ml) was placed in a 100 ml round-bottom flask equiped with magnetic stirr, esterification splitter and reflux condenser. The mixture was stirred and boiled to remove water. When drops of water ceased to appear in the splitter, the reaction was stopped. The ethynol and cyclohexane was evaporated and the residue was washed three times with cold saturated sodium chloride. The oily liquid obtained was dried with anhydrous magnesium sulfate for 2 h. The residue was distilled under reduced pressure to give ethyl 2-mercaptoacetate (7 g, 33%), a colorless liquid, bp 72 °C.

Synthesis of EDOTA To a 150 ml three-necked flask was added 10% sodium hydroxide (40 ml), ethyl 2-mercaptoacetate (10 g, 0.083 mol), tetrahydrogeranyl bromide (16.7 g, 0.076 mol) and few phase transfer catalyst tetrabutyl ammonium iodide, and stirred for 24 h. The upper yellowish liquid was separated and washed with saturated sodium chloride. The organic layer was dried with anhydrous magnesium sulfate for 2 h, then filtered and distilled under reduced pressure to give EDOTA (6.6 g, 33%), a light yellow liquid, bp 122—124 °C (5 mmHg).[6] The percentage purity was 90.10% by GC. IR (neat) cm^{-1}: 2940 (C-H), 1730 (C=O), 1460 (C-H), 1270 (S-C), 1130, 1030 (C-O-C). ^{1}H-NMR (CCl_4) δ: 0.88 (9H, d, J=6.1 Hz, $3CH_3$), 1.37 (13H, m, 2CH, $4CH_2$, $1CH_3$), 2.617 (2H, t, J=6.4 Hz, CH_2), 3.409 (2H, s, CH_2), 4.20 (2H, q, J=6.5 Hz, CH_2). MS m/z 260, 262 (M+2),

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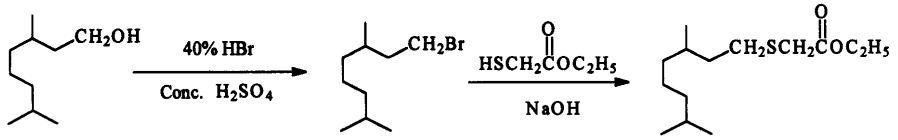

Chart 1. Synthesis of EDOTA

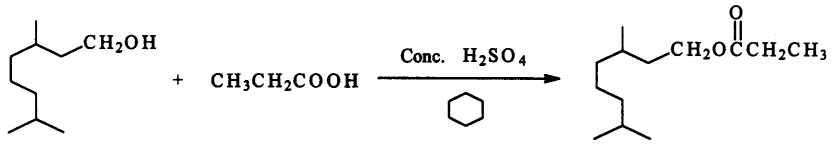

Chart 2. Synthesis of DOP

173, 83, 70.

Synthesis of DOP To a 100 ml round-bottom flask was added THG (12.5 g, 0.063 mol), propionic acid (7 g, 0.095 mol), cyclohexane (30 ml) and concentrated sulfuric acid (1.5 ml). The reaction mixture was stirred and refluxed for 5 h. The mixture was cooled and the organic layer washed initially with water, then with 5% sodium bicarbonate following drying with anhydrous magnesium sulfate for 2 h. The liquid was filtered and distilled under reduced pressure. The distillate collected at 106—108 °C (8 mmHg) gave DOP (10.5 g, 77.5%), a colorless and transparent liquid.[6] The percentage purity was 98.5% by GC. The structure of the product was confirmed by: IR (neat) cm^{-1}: 2940 (C-H), 1730 (C=O), 1460 (C-H), 1180, 1080 (C-O-C). ^{1}H-NMR ($CDCl_3$) δ: 0.86 (9H, d, J=6.1 Hz, $3CH_3$), 1.42 (13H, m, $4CH_2$, 2CH, $1CH_3$), 2.06 (2H, q, J=6.8 Hz, CH_2), 3.55 (2H, t, J=6.5 Hz, CH_2), MS m/z 215 (M+1), 185, 57.

***In Vitro* Percutaneous Permeation Experiments** Percutaneous permeation was investigated using *in vitro* permeation cells (Franz type). Full-thickness abdominal skins of male Sprague Dawley rats weighing 200—250 g were used. The hair of the abdominal area of rats was removed with clippers without damaging the underlying skin. The skin was excised from the abdomen, subcutaneous fat and other extraneous tissues were trimmed, and it was floated on normal saline for 12 h before use to ensure full tissue hydration. Generaly, hydration of SC, the principal barrier to transdermal drug delivery, is believed to enhance the rate of percutaneous absorption. Fully hydrated skin was mounted between donor and receptor cells with an available diffusion area of 2.01 cm^2. The receptor cell was filled with 16.5 ml normal saline, maintained at 37±0.5 °C and stirred at 400 rpm. A saturated solution of 5-FU at 32 °C was kept for 8 h in a thermostatically-controlled mechanical shaker and 1 ml was applied to the skin surface in the donor compartment and exposed to ambient temperature. At appropriate times, 3 ml solution was withdrawn from the receptor compartment and 1 ml was used for analysis. After sampling, 3 ml fresh normal saline was added to the receptor cell to keep the volume constant. This dilution was taken into account when evaluating the permeation data. Permeation experiments were carried out for 24 h.

To determine the enhancing effect of EDOTA, DOP, THG and Azone, the skins were prepared in the same way and mounted between donor and receptor cells, but, in this case, the skin was treated with 150 μl EDOTA, DOP, THG or Azone and the donor cell was covered with Parafilm to prevent evaporation. After 12 h, excess enhancer was removed and replaced with 1 ml saturated 5-FU. Samples were withdrawn at predetermined time intervals and analyzed for drug.

The concentration of 5-FU in the diffusion media was measured spectrophotometrically. A double-beam spectrophotometer was used at a wavelength of 266 nm. Standard curves for 5-FU were constructed using the concentration *versus* absorbance values of 5-FU solutions of various concentrations in 10 ml water containing 1 ml filtered skin eluate. The calibration curves were constructed by linear regression fitting to give: Y=5.540(C)−0.01235; r=0.9998. The samples withdrawn were filtered through a 0.8 μm filter to reduce the possibility of contamination by subcutaneous fat and other visceral debris, and 1 ml was used for analysis. Blank samples were also run simultaneously throughout the experiment to check for any interference.

At the end of the experiment, the amount of 5-FU in the skin was determined as described by Tenjarala and Borazani.[7] The exposed skin was cut, the surface washed carefully with water to remove excess drug on the surface, blotted dry, weighed, cut into small pieces. The tissue was homogenized three times with 4 ml ethyl acetate. The homogenate were mixed, filtered under vacuum and the ethyl acetate evaporated to dryness. The residue obtained was reconstituted with 10 ml water and analyzed for the drug spectrophotometrically. All the permeation parameters, flux (J), permeability coefficient (K_p), enhancement ratio (ER), partition coefficient (P_c), diffusion coefficient (D), partition ratio (PR) and diffusion ratio (DR) were determined in the same manner as described in our previous report.[5]

Results and Discussion

EDOTA was synthesized by reacting tetrahydrogeranyl bromide with ethyl 2-mercaptoacetate in the presence of 10% sodium hydroxide solution and a phase transfer catalyst with stirring due to the heterogenerous reaction. This may well lead to partial hydrolysis of ethyl 2-mercaptoacetate under basic conditions, so the yield was also affected. Similar reactions with alkyl bromide which is very active gives only about 40% yield. Chart 1 represents the steps involved in the synthesis of EDOTA. Chart 2 shows the synthesis of DOP, which was prepared by reacting THG with propionic acid in the presence of cyclohexane and concentrated sulfuric acid. This is a common esterification reaction, because the difference in boiling points of THG and DOP was very small. The amount of propionic acid was so great that all the THG was used up and post-treatment was easy. The chemical structures of the synthesized derivatives were verified by IR, ^{1}H-NMR and MS.

The structures of the enhancers examined in this investigation are shown in Fig. 1. All these compounds are oils at room temperature. The time course of 5-FU permeation across untreated and enhancer-pretreated rat skin is shown in Fig. 2 and the corresponding permeation parameter: fluxes, permeability coefficients and enhancement ratios are summarized in Table 1. Drug penetration through untreated skin was very low. The cumulative amount found in the receptor compartment usually displayed a linear relationship with time, and flux was taken as the slope of the straight line. A steady-state skin flux was attained within a few hours of application of the drug solution. Moreover, no significant drug depletion from the donor compartment was observed and the steady-state skin flux was maintained over the entire period of the diffusion experiments for EDOTA and DOP. It is important to note that a saturated drug solution was used to ensure unit thermodynamic activity throughout the diffusion experiments. The cumulative amount of 5-FU that permeated over 24 h was 2.14 and 3.84 mg/cm^2 with EDOTA and DOP respectively and 0.39 mg/cm^2 without enhancers. The mean control value for the K_p of 5-FU in the untreated skin at 37±0.5 °C is $1.45\pm0.10\times10^{-3}$ cm/h. Steady-state permeation was observed for 24 h with EDOTA and DOP, probably

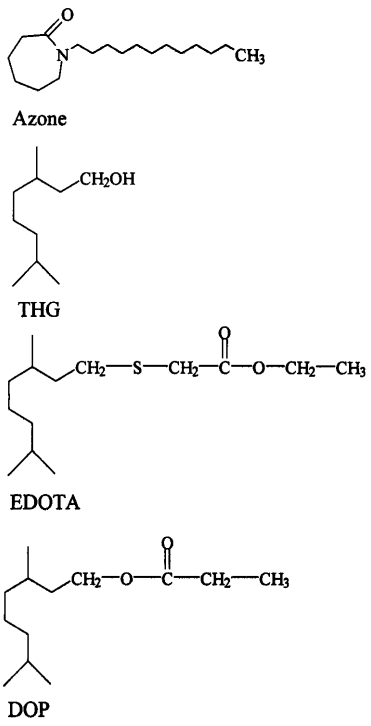

Fig. 1. The Structures of Azone, THG, and the New Derivatives

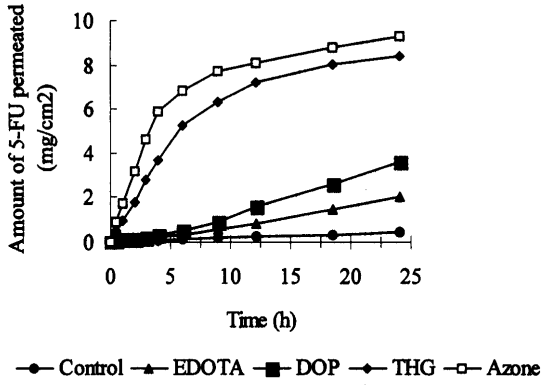

Fig. 2. The Penetration Profiles of 5-FU across Full-Thickness Rat Skin in the Absence and Presence of EDOTA, DOP, THG, and Azone

Each point shows the mean±S.D. of 4 to 7 experiments.

due to their slow penetration into skin. THG and Azone were used for comparison and showed steady-state permeation of 6 and 4 h, respectively. The penetration-enhancing activity of the enhancers is more clearly seen in terms of enhancement ratios. EDOTA and DOP increased the permeation of 5-FU was about 6-fold and 11-fold, respectively, while with THG and Azone, 5-FU permeation was about 48-fold and 83-fold respectively.

The partitioning results with these enhancers are shown in Table 2. Treatment of the skin with enhancers increased the partitioning of the drug into skin as illustrated by the partition ratio (PR),[8] where

$$PR=\frac{\text{partition coefficient after enhancer treatment}}{\text{partition coeffient with untreated skin}}$$

From the diffusion coefficient values (Table 2), it can be seen that all the enhancers reduced the resistance to diffusion of the drug as the mean untreated apparent diffusion coefficient value of 5-FU is only $0.13\pm0.02\times10^{-3}$ cm^2/h. The increase in diffusivity of 5-FU in the skin may be expressed as a diffusion ratio (DR),[8] where

Table 1. The Mean Flux (J), Permeability Coefficient (K_p) and Enhancement Ratio (ER) of 5-FU in Excised Rat Skin at 37±0.5 °C, before and after Treatment with EDOTA, DOP, THG, and Azone (n=4—7, Mean±S.D.)

Enhancers	Flux μg/cm^2/h	K_p cm/h$\times10^{-3}$	ER
Control	18.13±1.35	1.45±0.10	—
EDOTA	102.62±5.41	8.21±0.43	5.67±0.30
DOP	194.93±20.12	15.59±1.61	10.76±1.11
THG	875.14±70.14	0.01±5.60	48.30±3.87
Azone	1498.82±90.09	119.91±7.21	82.72±4.97

Table 2. Estimated Mean Apparent Partition Coefficient (P_c), Diffusion Coefficients (D), Partition Ratio (PR) and Diffusion Ratio (DR) of 5-FU into Fully Hydrated Excised Rat Skin (n=4—7, Mean±S.D.)

Enhancers	P_c $\times10^{-3}$	D cm/h$\times10^{-3}$	PR	DR
Control	34.43±4.70	0.135±0.02	—	—
EDOTA	55.35±4.70	0.45±0.06	1.61	3.34
DOP	68.42±9.52	0.69±0.09	1.99	5.12
THG	65.36±4.28	2.89±0.55	1.90	21.50
Azone	92.03±6.54	3.93±0.47	2.70	29.21

$$DR=\frac{\text{diffusivity of 5-FU in membrane after enhancer treatment}}{\text{diffusivity of 5-FU in membrane before enhancer treatment}}$$

Generally, the skin is considered to be a heterogeneous structure, composed of a comparatively lipophilic SC and hydrophilic viable skin (epidermis and dermis). Therefore, for hydrophilic penetrants, partitioning into the SC becomes the rate-determining step of skin permeation.[9] Partitioning and diffusion are directly affected by the molecular characteristics of the penetrants, such as solubility, size and shape.[10]

The present study was carried out to synthesize and investigate the penetration-enhancing effect of THG derivatives, EDOTA and DOP, on the permeation of 5-FU through excised rat skin. Significant differences were found in flux values of 5-FU using these enhancers. EDOTA and DOP increase the penetration of drug about 6 and 11 times, respectively, while THG and Azone enhanced the transdermal penetration of 5-FU about 48 and 83 times, respectively. The partition ratios calculated suggest that these enhancers increase the partition of 5-FU into the skin. As the drug is less soluble in enhancers than in water, this increased partition of drug may be due to the retention of the drug by skin, since in this study full-thickness skin was used to determine the drug. Therefore, we conclude that the enhanced permeation of 5-FU may not be due only to increasing the partition of drug into the stratum corneum but also to modifying the intercellular lipids, thus increasing the diffusion of 5-FU through the membrane, as observed from the diffusion ratios (Table 2).

From the results obtained in this investigation, it is concluded that some new percutaneous absorption enhancers have the potential to enhance the percutaneous penetration of 5-FU. Although the enhancing effects of THG and its derivatives on 5-FU permeation apeared to be less than that of Azone, the natural products may offer a large selection of relatively safe penetration enhancers to aid topical drug delivery. Additional experiments are required to determine where

in the skin these enhancers act and in what way they alter skin permeability.

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