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STUDY OF TOLNAFTATE RELEASE FROM FATTY ACIDS CONTAINING OINTMENT AND PENETRATION INTO HUMAN SKIN *EX VIVO*

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Abstract: Five fatty acids (oleic, linoleic, myristic, lauric and capric) were incorporated in 10% (w/w) into ointment formulation and their influence on lipophilic model drug tolnaftate release *in vitro* and enhancing effect on tolnaftate penetration into epidermis and dermis of human skin *ex vivo* were investigated. The prepared ointments were tested for homogeneity, pH and rheological properties. *In vitro* release studies and *ex vivo* skin penetration experiments were carried out using Hanson and Bronaugh-type flow-through diffusion cells, respectively. Tolnaftate cumulative amount liberated from semisolids was assayed using UV-Vis spectrophotometer. After *in vitro* skin penetration studies, appropriately extracted human skin layers were analyzed for tolnaftate content using a validated HPLC method. Statistical analysis revealed that release rate of tolnaftate from control ointment and ointments with fatty acids was not significantly different and only 7.34–8.98% of drug was liberated into an acceptor medium after 6 h. Tolnaftate amount penetrating into 1 cm^2 of epidermis from ointments containing oleic, linoleic, myristic and lauric acids was significantly greater ($p < 0.05$) than from the control ointment. Penetration enhancing ratios for these fatty acids for tolnaftate penetration into epidermis ranged from 1.48 to 1.75. In conclusion, fatty acids did not increase the liberation of tolnaftate from ointment formulation, but demonstrated their enhancing effect on tolnaftate penetration into human epidermis *in vitro*. Results from *in vitro* release experiments do not suit for prediction of the situation in the skin *in vitro*, if chemical penetration enhancers are incorporated into the ointment formulation.

Keywords: penetration enhancers, fatty acids, *in vitro* skin penetration, *in vitro* release, tolnaftate

Intact *stratum corneum* (SC), the outermost layer of the skin, functions as the main barrier for the penetration to and permeation through the skin for drug molecules. In the SC, corneocytes are surrounded by continuous matrix of neutral lipids. Independently of the molecule passive diffusion by the polar transcellular or lipoidal intercellular route, it has to diffuse through the lipid layers in order to cross the barrier. Therefore, alteration of SC lipid organization by modifying the order of hydrophobic lipid tails influences the rate and extent of the diffusion process in the SC. This reversible alteration might be achieved by adding various lipophilic chemical penetration enhancers (CPEs), which are pharmaceutically acceptable, to the drug formulation.

The SC lipids, which are arranged into lamellar bilayers (1), are mainly composed of ceramides (40–50%), free fatty acids (10–15%) and cholesterol (25%) in an approximately 1 : 0.9 : 0.4 mol ratio (2–7). Lipophilic CPEs interact with lipid hydrocarbon chains, disrupt the order of lipid packing and increase fluidization in the bilayers. In this way CPEs are capable to locally enhance the penetration of active compounds into SC and through it (8).

Fatty acids belong to the lipophilic CPEs and their penetration enhancing properties are related to the hydrocarbon chain length and the presence of double bonds. Obviously, the vehicle in which the CPE is dissolved also influences the magnitude of enhancement (8). Oleic acid is considered to be the most potential CPE among fatty acids due to its *cis*-

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monounsaturation and „kink“ formation (2). Oleic acid creates a highly permeable, fluid-like phase within the SC lipids (9).

Tolnaftate was chosen as a model drug for the investigation of the enhancing effect of five fatty acids (oleic, linoleic, myristic, lauric and capric) on its penetration from 1% (w/w) ointment formulation into epidermis and dermis of human skin after *in vitro* skin penetration experiments. Tolnaftate is a topical antifungal drug of thiocarbamate class, acting against dermatophytes, which mainly invade the superficial layers of the skin. Tolnaftate is a lipophilic compound (XLogP = 5.5) of low molecular weight (307.4 Da), having weak basic properties and melting point of 109–112°C (Eur. Pharm.6.0; 01/2008:1158).

MATERIALS AND METHODS

Chemicals and reagents

Tolnaftate (*O*-naphthalen-2-yl methyl(3-methylphenyl)thiocarbamate; Eur. Pharm. 6.0) with purity of 99.7% was a gift from pharmaceutical company Sanitas AB (Kaunas, Lithuania). Polyethylene glycol 400 (PEG 400) was purchased from Carl Roth GmbH (Karlsruhe, Germany). Polyethylene glycol 1500 (PEG 1500) and capric acid (decanoic acid, C10:0) were obtained from Merck Schuchardt OHG (Hohenbrunn, Germany); white vaseline, anhydrous lanolin, oleic acid (*cis*-9-octadecenoic acid, C18:1) and methanol (Chromasolv®) were purchased from Sigma-Aldrich Chemie GmbH (Steinheim, Germany). Ethanol (96.3%) was obtained from Stumbras AB (Kaunas, Lithuania). Linoleic acid (*cis,cis*-9,12-octadecadienoic acid, C18:2), myristic acid (tetradecanoic acid, C14:0) and lauric acid (dodecanoic acid, C12:0) were purchased from Alfa Aesar GmbH (Karlsruhe, Germany). Sodium azide (NaN_3) was obtained from POCh (Gliwice, Poland). All other reagents were of analytical grade.

Human skin preparation

Caucasian women (of age 25–40) abdominal skin was obtained after cosmetic surgery in the Department of Plastic and Reconstructive Surgery, Hospital of Kaunas University of Medicine. The studies with human skin were approved by Kaunas Region Bioethical Committee. The appropriately treated skin was wrapped in aluminum foil and stored at –20°C for not longer than 6 months before use.

Equipment

The prepared ointments were evaluated microscopically, using Motic® B3-series (Meyer Instruments, Texas, USA) with integrated Moticam 1000 digital microscope camera. pH values and rheological properties were assessed using portable pH-meter HD2105.1 (Delta OHM, Italy) and rotary viscometer ST-2010 (JP Selecta S.A., Spain), respectively.

In vitro release studies were carried out using an assembly constructed in our laboratory. Hanson vertical diffusion cells, having semisolid formulation deposited in the donor chamber and covered by Cuprophane® cellulose-dialysis membrane (Medicell International Ltd., London, U. K.), were inserted into the glass beaker and overlayered with an appropriate acceptor medium. The glass beaker was put into the thermostated water bath (Grant GD120, Grant Instruments Ltd., Cambridge, Great Britain) and efficient mixing of the acceptor medium was achieved using Heidolph RZR 2021 mechanical stirrer with propeller-type impeller (Heidolph UK, Essex, U. K.).

The equipment for *in vitro* skin penetration experiments consisted of thermostated water bath (Grant GD120, Grant Instruments Ltd., Cambridge, Great Britain), peristaltic pump (Masterflex® L/S® pump drive (model type 7524-45) with multichannel pump head (model type 07535-08), Cole-Parmer Instrument Co., Illinois, USA) and Bronaugh-type flow-through diffusion cells, made from Teflon. Extraction procedure of human skin layers was performed in the Bandelin Sonorex Digitec Ultrasonic Bath (DT 156, Bandelin electronic GmbH & Co. KG, Berlin, Germany), having ultrasonic peak output of 640 W.

UV spectrophotometric analysis was done using UV-Vis Unicam Helios-α spectrophotometer (Unicam, Analytical Technology Inc., Cambridge, Great Britain).

High performance liquid chromatography (HPLC) analysis was carried out using Shimadzu Liquid Chromatograph (Shimadzu Corporation, Kyoto, Japan) equipped with two Shimadzu LC-10AD VP pumps, degasser DGU-14A, auto injector SIL-10AD VP, system controller SCL-10A VP, UV-Vis detector SPD-10A and column oven CTO-10AC VP.

Preparation of ointments

One percent (w/w) tolnaftate ointments were prepared by dissolving tolnaftate in PEG 400 and incorporating the solution into the absorption base (fusion technique). The absorption base was composed of white vaseline, anhydrous lanolin and PEG 1500. These components were melted together (70°C) on a steam bath and the hot (70°C) solution

of tolnaftate in PEG 400 was slowly added. Stirring was maintained until congealed.

Ointments having 10% (w/w) of different fatty acids were prepared in the same manner as the control ointment except that an appropriate amount of fatty acid was melted together with the components of the absorption base (myristic, lauric and capric acids) or poured into the molten phase (oleic and linoleic acids). The composition of the ointments prepared is presented in Table 1.

Physico-chemical properties of prepared ointments

Homogeneity, pH and rheological properties of prepared ointments were evaluated. For homogeneity assessment, the ointments were smeared on the glass slide and examined under the microscope. For pH evaluation, each ointment (2.5 g) was heated (50°C) in 50 mL of distilled water and stirred on the magnetic stirrer for 50 min. Cooled solutions were filtered through ashless filter paper (Albet® 145, pore diameter 7–11 μm) and their pH was evaluated using pH-meter. Dynamic viscosity η (Pa·s) of each ointment was assessed at ambient temperature (21°C) using rotary viscometer coupled with cylindrical spindle (R7), rotating in the selected speed interval of 1.5–10.0 rpm (each measurement lasted for 50 s, n = 3).

***In vitro* release studies**

In vitro release tests were carried out using Hanson vertical diffusion cells with effective diffusion area of 1.77 cm^2. Accurately weight amount of ointment (approximately 0.8 g) was spread evenly in the donor compartment and covered with the Cuprophan® cellulose-dialysis membrane, which was pre-hydrated in distilled water at 37°C for 24 h. Hundred milliliters of acceptor medium, composed of 50/50 PEG 400/96.3% ethanol, provided sink conditions (solubility of tolnaftate at saturation was determined to be 18.75 ± 1.06 mg/mL, n = 3). The acceptor medium was mixed at 100 rpm, using mechanical stirrer with propeller-type impeller. The whole assembly was kept in the water bath (37°C). Aliquots of 3 mL were collected after 1, 2, 4 and 6 h. After withdrawal of an aliquot, an equal volume (3 mL) of an acceptor medium (37°C) was immediately added to maintain a constant volume. The quantitative analysis of samples was performed using UV-Vis spectrophotometer.

***In vitro* skin penetration experiments**

Bronaugh-type flow-through diffusion cells, mounted with full-thickness human skin (the diffusional area being 0.64 cm^2) and having the receptor volume of 0.13 mL, were placed on the heating block (37°C). Twelve hours equilibration period was followed, circulating 0.9% NaCl + 0.005% NaN_3 underneath the skin. After the equilibration period, about 200 mg of the donor phase (infinite dose) was applied on the SC side of the skin surface for 24 h. The cells were covered with aluminum foil. The acceptor fluid, which was pumped at a rate of 0.6 mL/min by the peristaltic pump, was composed of 4 mL of 0.9% NaCl + 0.005% NaN_3 and it was entirely replaced after 4, 8 and 24 h. After 24 h, the donor phase was removed and the skin surface was rinsed 2 times with 0.5 mL of 96.3% ethanol and then 2 times with 1 mL of 0.9% NaCl. The outer residuals of skin samples were trimmed off, leaving the central circles with area of 0.64 cm^2.

Separation of skin layers and their extraction

Epidermis was separated from the rest of the skin (dermis) using dry heat separation method. The skin sample was placed (on the epidermis side) on the hot surface (60°C) for 1–2 s and epidermis was peeled off. Then, epidermis and dermis were separately extracted with 1 mL of pure methanol, following bath sonication for 30 min. The supernatant was filtered through nylon membrane filter (0.45 μm, Carl Roth GmbH, Karlsruhe, Germany) and injected into HPLC.

UV-Vis spectrophotometrical method

Samples obtained after *in vitro* release studies were analyzed using UV-Vis spectrophotometer. The absorbance peaks of tolnaftate were measured at λ = 257 nm and the calibration curve (in the concentration range of 1.5–7.5 μg/mL) was prepared in the acceptor medium, composed of 50/50 PEG 400/96.3% ethanol (R^2 = 0.9984, y = 59.77x + 0.4247). The UV spectrophotometric method was also used to quantify tolnaftate content remaining in the donor phase and skin washings, after *in vitro* skin penetration experiments. The donor phase was carefully removed, appropriately extracted (using 96.3% ethanol and sonication at 60°C for 30 min), filtered through the nylon membrane filter (0.45 μm) and analyzed according to the calibration curve (in the concentration range of 3.0–15.0 μg/mL) prepared in 96.3% ethanol (R^2 = 0.9948, y = 58.47x + 0.09089).

HPLC method

A validated HPLC method with UV detection was used to determine tolnaftate amount, penetrating into epidermis and dermis from 1% tolnaftate ointments after 24 h of topical application (10). The

Table 1. Composition of 1% (w/w) tolnaftate ointment, having 10% of fatty acid.

Tolnaftate	1.0
PEG 400	19.0
Fatty acid	10.0
PEG 1500	10.0
Anhydrous lanolin	15.0
White vaseline 45.0	
	100 g

Table 2. pH values of prepared ointments

1% (w/w) tolnaftate ointment	pH value
Control	5.91
Ointment with 10% oleic acid	4.34
Ointment with 10% linoleic acid	4.12
Ointment with 10% myristic acid	5.20
Ointment with 10% lauric acid	5.19
Ointment with 10% capric acid	4.65

Table 3. Cumulative amounts (%) of tolnaftate released after 6 h, mean release rates and determination coefficients (R^2) of lines, yielded by Higuchi-plots

Ointment	Cumulative amount of drug released after 6 h (% ± SD, n = 3)	Release rate ($\mu g/cm^2\sqrt{h} \pm SD$, n = 3)	R^2
Control	8.09 ± 1.07	161.09 ± 10.62	0.9949
With oleic acid	8.19 ± 0.23	189.50 ± 8.71	0.9879
With linoleic acid	8.98 ± 1.18	192.98 ± 10.92	0.9890
With lauric acid	8.49 ± 0.27	185.53 ± 18.17	0.9991
With capric acid	7.34 ± 0.51	168.05 ± 14.69	0.9987
With myristic acid	7.63 ± 0.07	166.26 ± 10.54	0.9933

Table 4. ERs of fatty acids for tolnaftate penetration into epidermis and dermis.

Fatty acid	ER (epidermis)	ER (dermis)
Oleic acid	1.75	1.24
Linoleic acid	1.62	0.99
Myristic acid	1.48	1.24
Lauric acid	1.62	1.30
Capric acid	1.09	1.13

samples of receptor fluid were also analyzed using HPLC.

Separation of tolnaftate from endogenous compounds, deriving from skin matrix, was accomplished on a LiChrospher 100 RP-18 endcapped column, 125 × 4 mm, i.d., packed with 5 µm size particles (Merck KGaA, Darmstadt, Germany). A LiChrospher 100 RP-18e (5 µm) (LiChroCART 4-4) was used as a guard column.

Isocratic elution, using 70% methanol and 30% bi-distilled water as the mobile phase, resulted in tolnaftate retention time of 13.2 min and the overall running time of 15.0 min. The flow rate of the mobile phase was set to 0.8 mL/min and the injection volume was 10 µL. The column was thermostated at 40°C and tolnaftate detection was set at λ = 257 nm.

Statistical analysis

For the statistical analysis, one-way analysis of variance (ANOVA) together with Tukey's HSD test were applied using SPSS software version 12.0. The level of significance was determined as $p < 0.05$.

RESULTS

Quality control tests of prepared ointments

The prepared ointments were all uniform in appearance, having light yellow color and an odor of oil (in case of oleic or linoleic acid) or a specific odor of saturated fatty acid added. Observation of prepared ointments under the microscope revealed, that no solid particles were present.

pH values of 1% (w/w) tolnaftate control ointment and ointments with fatty acids are presented in Table 2. Ointments with 10% fatty acids had lower pH values compared to the control, but the pH was appropriate for application of these ointments on the skin surface as pH of SC and upper viable epidermis is evaluated to be 4.0–4.5 and 5.0–7.0, respectively (11).

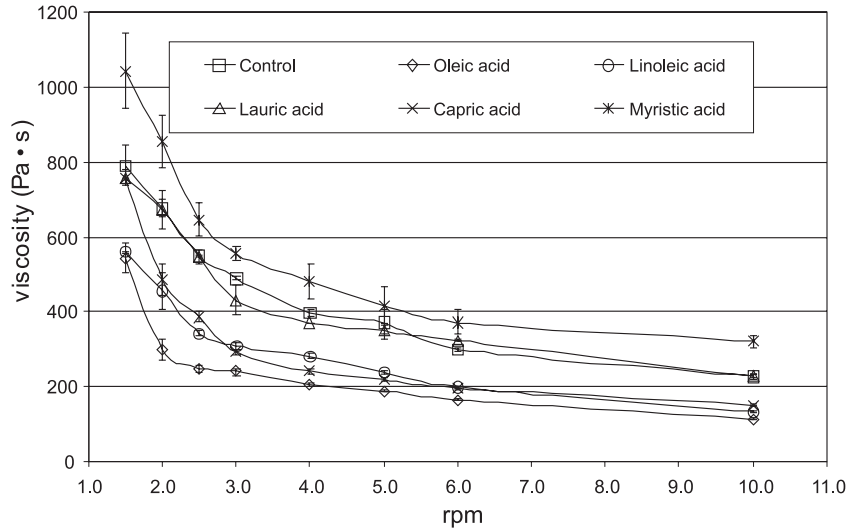

Figure 1. Relationship between ointments dynamic viscosity (Pa · s) and rotation speed of the spindle (rpm); the bars represent ± SD, n = 3

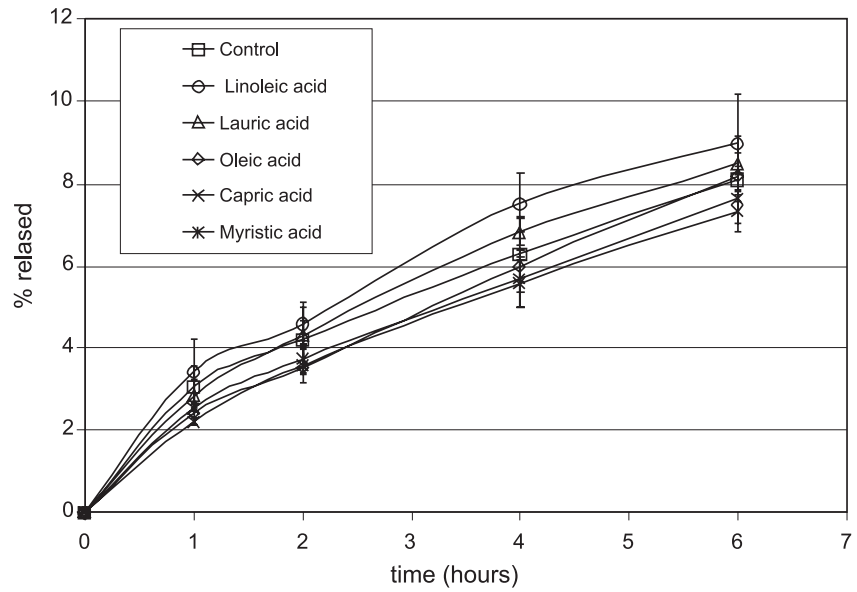

Figure 2. Percentage release of tolnaftate from ointment formulations; the bars represent ± SD

The rheograms, showing the relationship between dynamic viscosity and selected speed interval of cylindrical spindle rotation, are presented in Figure 1. The investigated ointments exhibited non-Newtonian pseudoplastic (shear-thinning) flow.

***In vitro* release profiles of tolnaftate from prepared ointments**

The percentage of the cumulative amount of tolnaftate released from each ointment after 1, 2, 4 and 6 h is presented in Figure 2. The cumulative amount (μg) of tolnaftate released per unit of membrane area (1 cm^2) was plotted against square root of time (Higuchi plot; Fig. 3). Higuchi plots yielded straight lines, the slopes of which indicated the release rates. Table 3 summarizes the parameters of tolnaftate release from prepared ointments *in vitro*, comprising the cumulative amounts (in %) of drug released after 6 h, release rates and coefficients of determination (R^2). The linearity (expressed as R^2 in

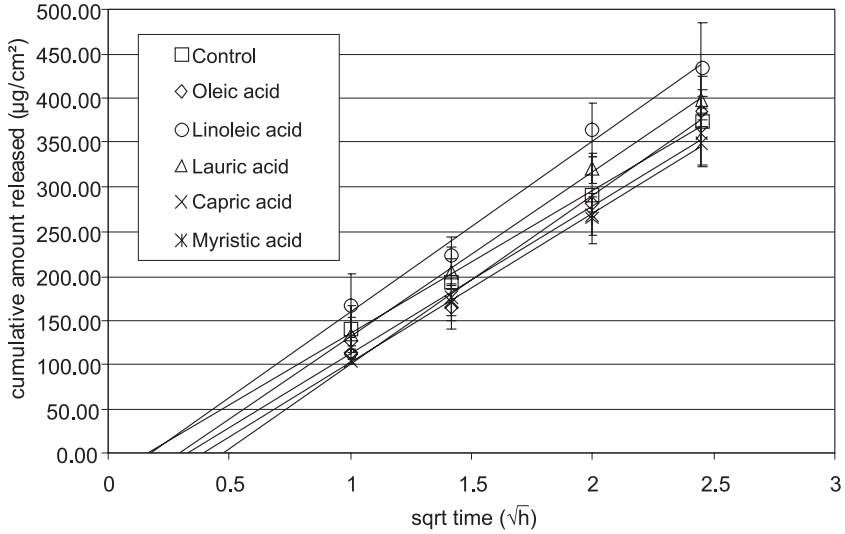

Figure 3. Higuchi plots of tolnaftate release from ointments; the bars represent ± SD

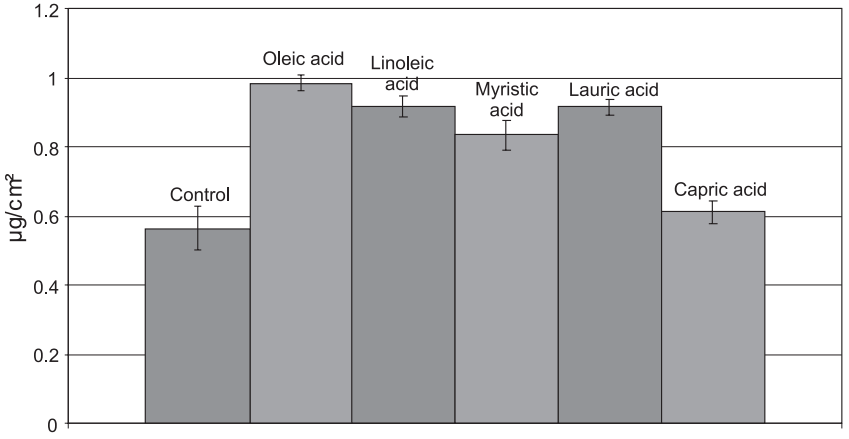

Figure 4. Tolnaftate amount (µg/cm^2) penetrating into epidermis from control ointment (n = 3) and ointments having 10% of fatty acids (n = 3). The bars represent ± SD

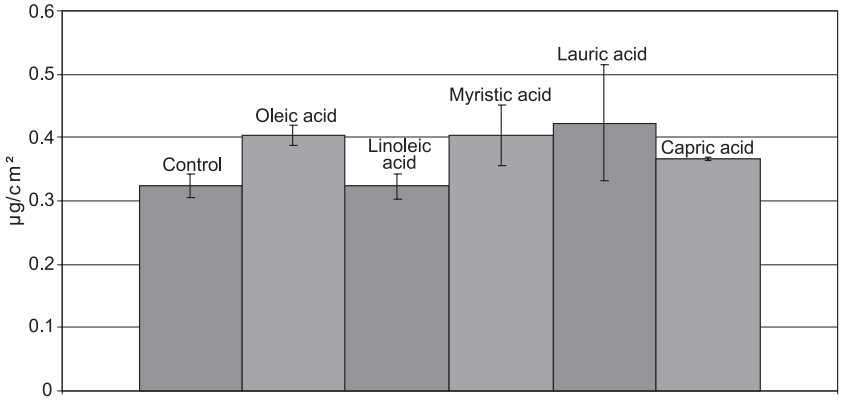

Figure 5. Tolnaftate amount (µg/cm^2) penetrating into dermis from control ointment (n = 3) and ointments having 10% of fatty acids (n = 3). The bars represent ± SD

Table 3) of Higuchi plots indicates, that diffusion of tolnaftate from ointments is a rate limiting step for drug liberation. Moreover, it shows, that Cuprophan® membranes used were not limiting drug diffusion from the donor to receptor compartment. The lag time for drug release, corresponding to x intercepts of the Higuchi-plots, were not exceeding 15 min for all ointments tested.

ANOVA showed that the release rate of tolnaftate from ointments was not significantly different ($p = 0.05$).

Enhancing effect of fatty acids on tolnaftate penetration into human skin layers

Figures 4 and 5 present the amounts ($\mu g/cm^2$) of tolnaftate penetrating into epidermis and dermis, respectively, from the control ointment (n = 3) and from ointments having 10% (w/w) of different fatty acids (n = 3) after *in vitro* skin penetration experiments. The enhancing effect of five different fatty acids on tolnaftate penetration into 1 cm^2 of epidermis (E) or dermis (D) was calculated as the enhancing ratio (ER) using the following formula:

$$ER = \frac{T \text{ amount in E or D from formulation with fatty acid } (\mu g/cm^2)}{T \text{ amount in E or D from control formulation } (\mu g/cm^2)}$$

The obtained ER values are presented in Table 4.

Statistical analysis revealed that tolnaftate amount penetrating into cm^2 of epidermis from the ointments having 10% of oleic, linoleic, myristic and lauric acids was significantly greater ($p < 0.05$) than from the control ointment. Capric acid did not significantly enhanced tolnaftate penetration into epidermis comparing to the control. According to Tukey HSD test, three homogenous subsets of groups whose means do not differ from one another, were found: ointments with (i) oleic, linoleic and lauric acid, (ii) linoleic, myristic and lauric acid, (iii) capric acid and control.

High amounts of tolnaftate in hydrophilic dermis could not be expected, as tolnaftate is a hydrophobic substance. Tolnaftate penetration into dermis was not significantly enhanced from either ointment having 10% of fatty acid comparing to the control ($p = 0.05$). These findings prove that fatty acids exert their enhancing effect only in the superficial layers of skin.

Mass balance of tolnaftate after *in vitro* skin penetration studies

In order to check the experimental conditions used for *in vitro* skin penetration experiments, mass balance of tolnaftate was calculated. After *in vitro* skin penetration experiments, the donor phase, remaining on the skin surface, was carefully removed, appropriately extracted with 96.3% ethanol and analyzed by UV-Vis spectrophotometry. The tolnaftate amounts remaining in the donor phase and found in the epidermis and dermis extracts were summed up (no drug was detected in the acceptor fluid) and compared to the nominal amount of tolnaftate added on each skin sample. The relative error (RE) was calculated according to the formula:

$$RE(\%) = \frac{(\text{found amount of } T - \text{true amount of } T)}{\text{true amount of } T} \times 100$$

The REs were calculated for each skin sample and were in the range from –4.19% to 8.45%. This certifies adequately chosen experimental conditions.

DISCUSSION

White vaseline was chosen as the main vehicle component for model drug tolnaftate ointment formulation. Other ingredients were added to improve formulation's consistency, spreadability, appearance and tolnaftate solubility. PEG 400 was chosen as an appropriate solvent for tolnaftate. Tolnaftate 1% solution and creams, available over-the-counter, are usually formulated in PEG 400 or PEG 400 – propylene glycol vehicle, respectively. PEG 1500 was added as a plasticizing and thickening agent. Anhydrous lanolin acted as emulsifier. Fatty acids were chosen as potent CPEs for a hydrophobic tolnaftate. It is worth mentioning that polyethylene glycols are also considered to be good CPEs, thus synergistic enhancing action of PEGs and fatty acids could be expected (12). Model drug tolnaftate was completely dissolved in the prepared ointments.

The pH values of prepared ointments were compatible with the skin's surface pH. Dynamic viscosity measurement of prepared ointments revealed that semisolid preprations exhibited non-Newtonian shear-thinning flow. Addition of solid fatty acids (capric, lauric, myristic) obviously resulted in higher dynamic viscosity range comparing to the ointments with oleic or linoleic acid. In the control ointment formulation, 10% of fatty acids were replaced by the appropriate quantity of white vaseline. Only formulation with 10% myristic acid had higher dynamic viscosity range comparing to the control.

Drug release from a semisolid dosage form is a necessary prerequisite for drug availability for the skin and is evaluated using *in vitro* release tests. These tests characterize the performance of the vehicle in drug solubility, diffusion and release processes. The chosen acceptor medium (50/50 PEG 400/96.3% ethanol) provided sink conditions and

the cellulose membrane with nominal molecular weight cut-off of 10 kDa was not limiting drug diffusion. Moreover, Cuprophan® membranes are recommended by USP 30 for transdermal delivery system release studies. About 0.8 g (0.45 g/cm^2) of the product were applied to the donor chamber and this establishes infinite dose conditions (13).

The liberation of drug from semisolid formulation mainly depends on (i) the solubility of drug in the formulation and (ii) the viscosity of semisolid. Tolnaftate was soluble in the ointments prepared by fusion technique. An increase in the viscosity usually results in lower release of drug from semisolid. According to the results obtained, addition of liquid fatty acids (oleic and linoleic acid) to the ointments, resulted in higher release of tolnaftate, and this might be due to a decrease of viscosity of the formulation and possibly due to solubilizing effect of these fatty acids on hydrophobic tolnaftate.

ANOVA test showed that there is no significant difference among the release rates of tolnaftate from ointment formulations ($p = 0.05$). It means that fatty acids tested do not significantly affect the liberation of tolnaftate from ointment formulations. The enhancing effect of CPEs tested is only observed after making *in vitro* skin penetration experiments. Similar observations were made by Wagner et al. (14) after investigation of CPEs effect on the liberation and penetration of flufenamic acid. Our results indicated significantly higher penetration of tolnaftate molecules to epidermis from ointment formulations having 10% of oleic, linoleic, lauric and myristic acid comparing to the control. This might be explained by the insertion and interaction of fatty acid molecules with the lipid domains in SC and the increase of fluidization and disruption of lipid structure in the superficial layers of skin. Tolnaftate penetration into hydrophilic dermis was not significantly enhanced by fatty acids tested compared to the control.

Fatty acids increase the penetration of active drugs into the skin through the non-polar route, which is very important for the penetration of hydrophobic drugs, such as tolnaftate. The formulation of ointment itself, being the lipophilic base, has occlusive effect on the skin. Thus, skin hydration might be increased also resulting in higher penetration of active molecules.

The effect of fatty acid hydrocarbon chain length and presence of double bonds on its skin penetration enhancing properties was observed with our model drug tolnaftate. Among fatty acids tested, capric acid has the shortest chain length of C10 and this fatty acid did not significantly enhance the penetration of tolnaftate to epidermis comparing to the control. As stated by Kravchenko et al. (15), fatty acids having hydrocarbon chain length of less than C11, produce insufficient disruption of intercellular lipid alkyl radicals in the SC. Oleic and linoleic acids are unsaturated and have the longest chain length of C18. These two fatty acids were the strongest CPEs among the others tested, but oleic acid was the most potent CPE due to its *cis*-unsaturation and 'kink' formation (2), which is responsible for the fluidization of SC lipids (9, 16). Lauric acid (C12) together with oleic and linoleic acids belonged to the same homogenous subset (Tukey HSD test) and demonstrated very strong enhancing effect on tolnaftate penetration into epidermis. Similar results were obtained by Nair et al. after testing oleic, linoleic and lauric acids enhancing effect on arginine vasopressin (17). Lauric acid may have a spatial form that is conformationally similar to the framework of cholesterol (15). Thus Nair et al. (17) hypothesized that in the presence of lauric acid disruption of ceramide–cholesterol or cholesterol–cholesterol interaction might occur. Myristic acid, which has the chain length of C14, also significantly enhanced tolnaftate penetration into epidermis.

The ointments tested release tolnaftate in almost the same manner (about 8% of tolnaftate was released after 6 h from all formulations). The effect of the semisolid vehicle on the absorption kinetics of tolnaftate into the skin might be associated with the hydration process of skin surface (8). However, fatty acids, presented in the ointments, penetrated into the skin and interacted with the intercellular lipids of SC, disturbing their structure and enhancing the diffusion process of hydrophobic tolnaftate into human epidermis, but not dermis.

CONCLUSIONS

The formulated topical ointments, having 10% (w/w) of fatty acids, were intended to increase the bioavailability of tolnaftate in human skin layers *in vitro*. Addition of fatty acids to the ointments, having 1% (w/w) of tolnaftate as a model drug, does not significantly improve the liberation of active drug to the chosen acceptor medium compared to the control, but significantly enhances drug penetration into human epidermis. This observation indicates that fatty acids exert their effect only when they are applied on the skin, and consequently no presumptions could be made about the enhancing effects of CPE's on drug penetration into the skin after *in vitro* release experiments.

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