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Research paper

In vitro studies on release and human skin permeation of Australian tea tree oil (TTO) from topical formulations

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Abstract

Essential oils are widely used in pharmaceutical and cosmetic preparations e.g. as fragrance, active ingredient or penetration enhancer. However, reports on skin absorption are rare. Therefore, the aim of our study was to investigate the capability of terpinen-4-ol, the main compound of Australian tea tree oil (TTO), to permeate human skin. In static Franz diffusion cells permeation experiments with heat separated human epidermis were carried out using infinite dosing conditions and compared to liberation experiments. The flux values of three different semisolid preparations with 5% TTO showed the rank order semisolid O/W emulsion (0.067 μl/cm^2 h) > white petrolatum (0.051 μl/cm^2 h) > ambiphilic cream (0.022 μl/cm^2 h). In comparison to the flux value obtained with the native TTO (0.26 μl/cm^2 h), the flux values are remarkably reduced due to the lower amount of terpinen-4-ol. P_{app} values for cream ($2.74 \pm 0.06 \times 10^{-7}$ cm/s) and native TTO ($1.62 \pm 0.12 \times 10^{-7}$ cm/s) are comparable whereas white petrolatum ($6.36 \pm 0.21 \times 10^{-7}$ cm/s) and semisolid O/W emulsion ($8.41 \pm 0.15 \times 10^{-7}$ cm/s) demonstrated higher values indicating a penetration enhancement. No relationship between permeation and liberation was found.

Keywords: Tea tree oil; Franz diffusion cell; Human heat-separated epidermis; Drug liberation; Drug permeation; Terpinen-4-ol

1. Introduction

Tea tree oil (TTO) is an essential oil derived by steam distillation of leaves of the Australian native tea tree, *Melaleuca alternifolia* (Myrtaceae). TTO is a complex mixture of about 100 different compounds mainly monoterpenes and their corresponding alcohols. The main constituent of TTO is terpinen-4-ol (30–40%). Smaller quantities of structurally related substances, e.g. α-terpinene, γ-terpinene, terpinolene and α-terpineol, are also present along with monoterpenes such as α-pinene, β-pinene, *p*-cymene, limonene, and 1,8-cineole as well as sesquiterpenes such as aromadendrene, viridiflorene and δ-cadinene [1].

TTO is reputed to have several medicinal properties including antibacterial [1], antifungal [2,3], antiviral [4,5], antiinflammatory [6] and analgesic properties [7]. In recent years, it has especially gained popularity as a topical antimicrobial agent. It is used in a 5% semisolid O/W emulsion and cream, respectively, for topical treatment of acne and toenail onychomycocis in humans [8,9]. Furthermore, TTO is reported to exhibit an antiherpetic effect when applied in a 6% gel formulation [5]. TTO is also recommended as an antiseptic agent in denture and mouth washes, and for the treatment of furunculosis and vaginitis [7]. In skin care products it is marked for cleaning, healing, and relieving itching, hotspots, abrasions and other minor rashes and irritations.

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Because the skin behaves as a primary chemical, diffusional, and mechanical natural barrier of the body against the external environment, the permeation of TTO or its components into and through the skin is important for the efficacy of the topical applied essential oil. Percutaneous uptake of terpenes has been observed in man [10,11] and animal [12]. Nevertheless, our knowledge on the pharmacokinetics and permeation of topical applied essential oils through human skin remains fragmentary due to the lack of experimental data.

To improve the topical application of TTO, detailed knowledge on the dermal absorption of the components of TTO through human skin is essential. Therefore, the aim of the present work was to investigate the capability of terpinen-4-ol, the main compound of TTO, to permeate through human skin. Using a modified HET-Cam test system, a high irritant potential for native TTO has been shown by Reichling et al. [13]. In contrast to those findings drug preparations containing up to 10% TTO were not irritant. Hence, in order to study the influence of formulations on the *in vitro* TTO skin permeation, different TTO-containing preparations (ointment, cream, semisolid O/W emulsion) were prepared, and tested against pure TTO. For *in vitro* permeation experiments a static Franz diffusion cell with human heat-separated epidermis as barrier between the donor compartment and the acceptor phase was chosen. This diffusion cell is a well-established in vitro model to address skin absorption [14]. In addition, release profiles of terpinen-4-ol were determined to characterise the semisolid preparations of TTO.

2. Materials and method

If not further specified, all used substances were of highest analytical degree available and were used without further purification.

2.1. Australian tea tree oil (TTO)

Australian tea tree oil (TTO; *Melaleuca alternifolia*) was purchased from Alva, Wallenhorst, Germany. TTO was used as obtained. The major substances of the essential oil, α-thujene (0.8%), terpinen-4-ol (44.7%), α-terpineol (3.0%), γ-terpinene (22.5%), 1,8-cineol (4.2%), and α-terpinene (9.7%), were identified via GC/GC-MS. The chemical structure of terpinen-4-ol is pictured in Fig. 1.

Monoterpene standards terpinen-4-ol were purchased from Roth, Karlsruhe, Germany; 1,8-cineol, *p*-cymen, α-terpineol, α-terpinene, and γ-terpinene were obtained from Fluka, München, Germany.

2.2. GC-method

Australian TTO as well as the monoterpene standards were analysed as 1% solution in *n*-hexane containing tridecane as internal standard. GC was performed using a Carlo Erba GC 6000 chromatograph equipped with a Spectra Physic Integrator SP 4290. The GC column was a 30 m × 0.25 mm (i.d.) fused silica capillary column coated with OV 1 (phase thickness: 0.25 μm) and with He as carrier gas (flow rate: 2 ml/min); split 1:10. Temperature programm: the initial column temperature was 42 °C for 2.5 min. Subsequently, the temperature rate was programmed from 42 to 300 °C in two steps, first 4 °C/min up to 120 °C and than 10 °C/min up to 300 °C. Injector temperature: 250 °C; detector temperature: 300 °C; injection volume: 1 μl. The limit of detection for terpinen-4-ol was 30 ng.

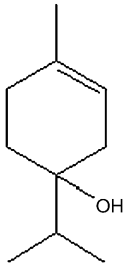

Fig. 1. Structure of terpinen-4-ol.

2.3. GC-MS methods

A gas chromatograph Carlo Erba MFC 500 was coupled via an open interface to a Finnigan MAT 4500 mass spectrometer. EI ionizing voltage 70 eV. GC column: OV-1, 30 m × 0.25 mm (i.d.). Initial column temperature: 46 °C for 4 min; temperature program: from 46 to 300 °C in three steps, first 3 °C/min up to 76 °C, then 4 °C/min up to 136 °C and 6 °C/min up to 300 °C. Components of the essential oil were identified by comparing their mass spectra with those of authentic samples.

2.4. Formulations

2.4.1. TTO ointment

Three concentrations of TTO were incorporated in white petrolatum: 3%, 5% and 10%. A microscopical inspection showed neither droplets nor phase separation in the preparation.

2.4.2. TTO cream

An ambiphilic cream consisting of Lanette O (10.0 P), Cera alba (5.0 P), glycerine (7.0 P), Lanette E (2.0 P), Paraffinum liquidum (16.0 P), Sorbinic acid (0.2 P) and Aqua destillata (ad 100.0 P) was chosen, containing 3%, 5%, and 10% TTO.

2.4.3. TTO semisolid O/W emulsion

Into aqueous polyacrylate gel, according to the German Pharmacopeia Nr. 10, 3% and 5% TTO were incorporated resulting in emulsions of O/W-type. For the 10% TTO phase separation occurred immediately after preparation and therefore this preparation was rejected.

2.5. Liberation experiments

The drug release experiments were carried out in static Franz diffusion cells (FD-C; type 6G-01-00-15-12; Perme Grear; Riegelsville, PA), where an Isopore® membrane with 0.05 μm pore diameter (Millipore, Eschborn) was positioned between the donor compartment, containing the drug preparation, and an acceptor compartment, filled with ethanol/water-mixture (1VP:1VP). The concentration of saturation of terpinen-4-ol in the ethanol/water-mixture was determined as 10.5 ± 3.6 μl/ml (mean ± SD). The temperature was kept at 32 ± 1 °C by a water jacket. The acceptor fluid was mixed with a magnetic stirring bar (400 upm).

Samples (0.8 ml) were drawn before each experiment and at regular time intervals from the middle area of the acceptor compartment and were immediately replaced with 0.8 ml fresh solution. In all cases sink conditions were maintained.

The time intervals for sample drawing were: 0.5, 1.0, 1.5, 2.0, 4.0, 6.0, 12.0, 18.0 h.

2.6. Skin dissection

Excised human skin from Caucasian female patients who had undergone abdominal plastic surgery was used. Approval from the Ethical Committee of the 'Caritas-Traegergesellschaft Trier e.v.', Trier, Germany, is on file. Immediately after excision the subcutaneous fatty tissue was removed using a scalpel. The skin was cut into 10 × 10 cm pieces, wrapped in aluminium foil and stored in polyethylene bags in a freezer at −26 °C until use. The maximum storage time was 6 months. Previous experiments had shown that neither the penetration characteristic nor the thickness of the SC (stratum corneum) was affected after a freezing period of 3 and 6 months, respectively [15].

For permeation experiments heat-separated human epidermis (HSE) was prepared according to Kligmann and Christophers [16]. Briefly, skin disks with a diameter of 35 mm were punched out, thawed, cleaned with cotton soaked with Ringer solution, and immersed in 60 °C hot water. After 60 s the skin was taken out of the water and placed on a filter paper (dermal side down). Subsequently, the SC and viable epidermis were carefully dissected from the dermis using forceps and transferred onto a Teflon disk. Afterwards, the HSE was stored in an exsiccator until use for a maximum of 7 days. Before use the HSE was rehydrated with phosphate buffer solution, pH 7.4, for at least 1 h. Previous experiments have shown that within this time the HSE is completely rehydrated (data not shown).

2.7. Permeation experiments

All permeation experiments were done using an infinite dosing regime [17]. For better handling the HSE was transferred onto a cellulose membrane (MC 10000; Medicell, London, UK) and carefully attached to each other. Afterwards this sandwiched membrane was gently pressed against the drug formulation in the donor compartment and donor and acceptor compartments were clamped together. The acceptor fluid was an ethanol/water-mixture (1VP/1VP) and kept at 32 ± 1 °C using a water jacket during the whole experiment. The acceptor fluid was mixed with a magnetic stirring bar (400 rpm). For both types of experiments (liberation and permeation) the same type of Franz diffusion cell was taken. Samples of 1.5 ml were drawn before each experiment and after predetermined time intervals from the middle area of the acceptor compartment and immediately replaced with 1.5 ml fresh solution.

2.8. Quantitative determination of terpinen-4-ol in the acceptor medium

For quantitative determination of terpinen-4-ol permeated in the acceptor phase of the permeation or liberation experiments an aliquot of the acceptor medium was extracted repeatedly with *n*-hexane. Subsequently, *n*-hexane phases were combined, tridecane as internal standard was added, and terpinen-4-ol was analysed quantitatively as described in section GC-method.

2.9. Calculations for liberation experiments

The amounts of terpinen-4-ol in the acceptor medium were corrected by the removed 0.8 ml of the samples.

According to the well-known Higuchi equation [18] for semisolid preparations with drug in solution

$$Q = A\ 2\ C_0\sqrt{(D\ t/\pi)}$$

with Q is the amount released, A is the diffusion area, C_0 is the drug concentration in the preparation, D is the diffusion coefficient, and t is the time, the amount of terpinen-4-ol released per diffusion area is plotted against the square root of time (Higuchi-plot). From the linear part of the Higuchi-plot the release rate was estimated by linear regression as slope [19].

2.10. Calculations for permeation experiments

The amount of terpinen-4-ol in the acceptor medium was corrected by the removed 1.5 ml of the samples. Permeation parameters were obtained from the cumulative amounts of terpinen-4-ol in the acceptor medium permeated per cm^2 versus incubation time plots. The steady-state flux (J), representing the absorption rate per unit area, was determined from the slope of the linear portion of the plots. In all experiments the same number of data points was taken to calculate the steady-state flux. The apparent permeability constant P_{app} ($cm\ s^{-1}$) was calculated according to Fick's first law of diffusion [20,21], based on the steady state flux J ($\mu l\ cm^{-2}\ s^{-1}$) and the applied drug concentration C_i ($\mu l\ cm^{-3}$) of the donor: $P_{app} = J/C_i$.

All calculations were done by using SigmaPlot 9.0 (SPSS Inc., Chicago, IL).

3. Results

3.1. Liberation of terpinen-4-ol from semisolid preparations

The release profiles of terpinen-4-ol, the main compound of TTO, from the cream preparation (Fig. 2a), the semisolid O/W emulsion preparation (Fig. 2b) and the ointment (Fig. 2c) fit the Higuchi equation for all investigated concentrations. As expected, the rank order of the release rates (Table 1) follows the same order than the applied concentrations of active ingredient (10% > 5% > 3%). Considering the various preparations differences are visible. For the preparation with 3% TTO the ranking of the release rates is cream (0.18 μl/cm^2 $\sqrt{h}$) < ointment (0.416 μl/cm^2 $\sqrt{h}$) < semisolid O/W emulsion (0.57 μl/cm^2 $\sqrt{h}$) with the cream demonstrating the lowest release rate. Comparing the 5% preparations the rank order is cream (0.356 μl/cm^2 $\sqrt{h}$) < semisolid O/W emulsion (0.659 μl/cm^2 $\sqrt{h}$) < ointment (0.778 μl/cm^2 $\sqrt{h}$). In this case the order of semisolid O/W emulsion and ointment has been inverted. For the drug level of 10%, the release rate for the ointment (1.58 μl/cm^2 $\sqrt{h}$) is 2.5 times greater than that of the cream (0.66 μl/cm^2 $\sqrt{h}$).

Table 1
Release rate of terpinen-4-ol from different semisolid preparations

Preparation	Release rate according to the Higuchi equation (μl/cm^2 $\sqrt{h}$) mean ± SE
Cream 3%	0.184 ± 0.007
Cream 5%	0.356 ± 0.010
Ointment 3%	0.416 ± 0.010
Semisolid O/W emulsion 3%	0.565 ± 0.012
Semisolid O/W emulsion 5%	0.659 ± 0.038
Cream 10%	0.663 ± 0.017
Ointment 5%	0.778 ± 0.017
Ointment 10%	1.581 ± 0.035

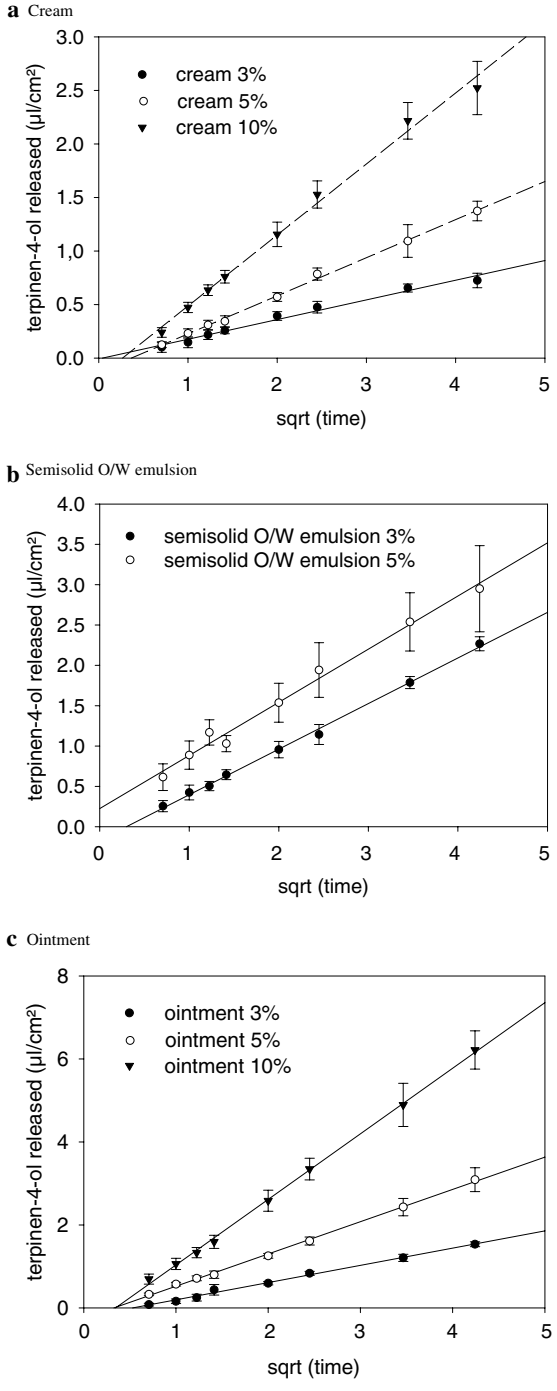

Fig. 2. Terpinen-4-ol liberation profiles of various TTO preparations (means ± SD; $n = 5$–6).

3.2. Permeation of terpinen-4-ol through heat-separated epidermis

The permeation profiles of terpinen-4-ol incorporated in TTO, cream 5%, semisolid O/W emulsion 5% and ointment 5% through heat-separated epidermis are shown in Fig. 3. The fastest permeation was found for the TTO followed by the semisolid O/W emulsion preparation, the ointment

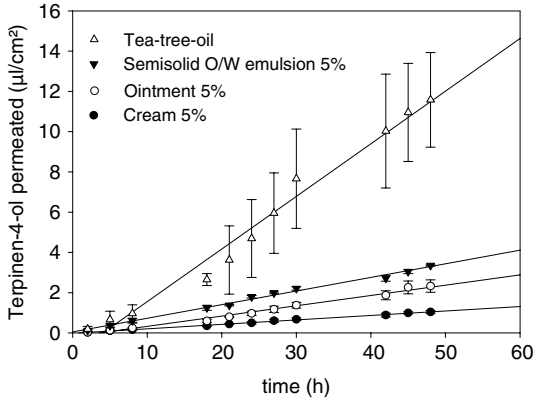

Fig. 3. Permeation of terpinen-4-ol through heat-separated human epidermis from various preparations (means ± SD; $n = 3$–4).

Table 2
Comparison of release and permeation data of terpinen-4-ol

Preparation	Content of terpinen-4-ol (μl/ml)	Release rate according to the Higuchi equation (μl/cm^2 $\sqrt{}$h) mean ± SE	Flux of terpinen-4-ol through heat-separated human epidermis (μl/cm^2 h) mean ± SE	P_{app} of terpinen-4-ol according to Fick's first law (cm/s) × 10^7 mean ± SE
Cream 5%	22.37	0.356 ± 0.010	0.022 ± 0.001	2.74 ± 0.06
Semisolid O/W emulsion 5%		0.659 ± 0.038	0.067 ± 0.001	8.41 ± 0.15
Ointment 5%		0.778 ± 0.017	0.051 ± 0.002	6.36 ± 0.21
Native tea tree oil	447.4	n.d.	0.262 ± 0.019	1.62 ± 0.12

and the cream. After a certain lag-time Fick's first law of diffusion is fulfilled leading to straight lines. The values for flux and apparent permeation coefficient of terpinen-4-ol were calculated according to Fick's first law of diffusion and are summarized in Table 2. Comparing the flux values of the three semisolid preparations the rank order is found to be semisolid O/W emulsion (0.067 μl/cm^2 h) > ointment (0.051 μl/cm^2 h) > cream (0.022 μl/cm^2 h). In comparison to the flux value obtained with the native TTO (0.26 μl/cm^2 h), the flux values of all semisolid preparations are remarkably reduced (0.02–0.07 μl/cm^2 h). Comparing the P_{app} values of the various formulations and the TTO itself, the differences between the cream and the native TTO were small but the values were lower than the ones of ointment and semisolid O/W emulsion which are nevertheless in the same range.

4. Discussion

In principle, liberation experiments allow the determination of the maximum release of an active ingredient from a semisolid preparation using the well-known Higuchi equation. Prerequisites are an infinite dosing, no rate limitation by the membrane separating donor and receptor compartment, and the receptor compartment acting as perfect sink. The maximum release depends on the physico-chemical and physical properties of the preparation and the active ingredient, e.g. solubility of the drug in the vehicle or viscosity of the formulation, and therefore allows the characterisation of complex semisolid preparations in a simple way in relation to their pharmaceutical quality. Information about interactions of the formulations with the skin will not be obtained from these experiments. However, if no release of the test compound will occur, it is assumed that no delivery to the skin will take place, either. Therefore, liberation experiments seem to be helpful tools in an early stage of the development of semisolid preparations especially highlighting interactions between excipients and drug release.

4.1. Liberation of terpinen-4-ol from the semisolid preparations

As could be expected from the Higuchi equation, all semisolid formulations demonstrated an increased release rate in dependence on the drug amount in the preparations (Fig. 2 and Table 1). The degree is differently pronounced by the various preparations. Considering the semisolid O/W emulsion preparations, both curves were almost parallel but starting from differing values (Fig. 2b). An explanation might be that those formulations showed a tendency for phase-separation and therefore, the same composition might be present at the border of the formulation to the membrane. This might also explain the reverse ranking of the semisolid O/W emulsion and the ointment for the 5% formulation. Comparing the three types of formulation (semisolid O/W emulsion / cream / ointment) the cream showed always the lowest release rate for all drug amounts under investigation. An explanation of this phenomenon might be the longer diffusion pathway, caused by the high dispersion of the two phases in the cream system which increases the tortuosity of the diffusion pathway. Besides the incorporation of terpinen-4-ol in the lipoidal phase building complex structures with the emulsifier system might play a role.

4.2. Permeation of terpinen-4-ol through heat-separated epidermis

The permeation of terpinen-4-ol through heat-separated human epidermis was clearly influenced by the applied formulation as illustrated in Fig. 3. For the TTO preparations the semisolid O/W emulsion demonstrated the highest flux value and P_{app} value followed by the ointment and the cream. Due to the high water content of the semisolid O/W emulsion the SC might be fully hydrated which was not achieved in a corresponding time period with the cream and the ointment. The importance of SC hydration was also outlined by Wagner et al. [22] for the lipophilic model drug flufenamic acid using aqueous polyacrylate gels in comparison with water-free ointments and W/O-creams. Furthermore, the instability of the semisolid O/W emulsion preparation might play a role by the formation of a thin layer of TTO on the surface of the heat-separated epidermis. For the good absorption of terpinen-4-ol from the ointment base co-diffusion of lipophilic compounds from the white petrolatum resulting in a change of the lipid composition of the SC might be responsible. Similar effects were reported by Jaeckle et al. [23] for ketoprofen. In contrast to the semisolid O/W emulsion and ointment the cream showed a reduced flux value and P_{app} value. The reason for this result could be the reduced release from this preparation.

As indicated in Fig. 3 and Table 2 terpinen-4-ol permeation from native TTO is higher in comparison to the preparations with 5% TTO. The reason for this behaviour is the higher content of terpinen-4-ol leading to increased flux values according to Fick's first law. Taking the different concentrations of terpinen-4-ol into account the apparent permeability coefficient P_{app} of terpinen-4-ol from the native TTO is very similar to those of the cream. Furthermore, the higher P_{app} values of the ointment and semisolid O/W emulsion in comparison to the cream and native TTO showed clearly that both preparations interacted with the skin by facilitating the permeation of terpinen-4-ol through heat-separated epidermis.

Comparing release rate and flux through heat-separated epidermis no relationship between both values could be identified. This result is in agreement with data reported by Wagner et al. [22]. As it is shown with the permeation experiments, terpinen-4-ol is delivered to the skin in reasonable amounts depending on the preparation used. Therefore, it can be assumed that TTO would be biologically available in the skin. Moreover, for steady state conditions Landvatter [24] has done a rough estimate of the terpinen-4-ol concentration in the epidermis for the preparations with 5% TTO (cream 2.03 mg/ml, semisolid O/W emulsion 0.91 mg/ml, ointment 18.04 mg/ml). Since these concentrations are higher than the minimal antibacterial concentration [1] the use of these preparations as disinfectant for pharmaceutical as well as cosmetically use is feasible. Furthermore, terpinen-4-ol is often found in various essential oils, e.g. juniper oil, lavender oil, rosemary oil, majoran oil, or thyme oil which are widely used in cosmetic preparations [25,26] or investigated as skin permeation enhancer [27,28]. Due to the multitude of applications and the high dermal availability the safety of preparations containing terpinen-4-ol must be scrutinised. Therefore, further experiments are needed to evaluate the influence of complex compositions of the formulations on skin permeation of essential oils.

5. Conclusion

The major compound of TTO, terpinen-4-ol, is able to permeate human epidermis very easily. The permeation depends on the applied preparation whereas a semisolid O/W emulsion or an ointment is superior to a cream. No relationship between liberation data and permeation values was found.

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