

Percutaneous Absorption of Salicylic Acid after Administration of Trolamine Salicylate Cream in Rats with Transcutol[®] and Eucalyptus Oil Pre-Treated Skin

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ABSTRACT

Purpose: This study was conducted to assess the effect of skin pre-treatment with Transcutol[®] and eucalyptus oil on systemic absorption of topical trolamine salicylate in rat. **Methods:** Pharmacokinetic parameters of salicylic acid following administration of trolamine salicylate on rat skin pre-treated with either Transcutol[®] or eucalyptus oil were determined using both non-compartmental and non-linear mixed effect modeling approaches and compared with those of control group. **Results:** Median (% of interquartile range/median) of salicylic acid AUC_{0-8hr} (ng/mL/hr) values in Transcutol[®] or eucalyptus oil treated rats were 2522(139%) and 58976(141%), respectively as compared to the 3023(327%) of the control group. Skin pre-treatment with eucalyptus oil could significantly decrease extravascular volume of distribution (V/F) and elimination rate constant (k) of salicylic acid. **Conclusion:** Unlike Transcutol[®], eucalyptus oil lead to enhanced transdermal absorption of trolamine salicylate through rat skin.

Introduction

Delivery of pharmacological agents via the skin provides distinct benefits compared to other conventional routes of administration, such as minimizing adverse effects and toxicity due to a steady and optimum blood levels, bypassing intestinal and hepatic first pass effect, prevention of gastrointestinal irritation, etc.¹⁻³ However, transdermal absorption of most drugs often results in a low bioavailability because of the barrier nature of the skin. The most important reason of resistance to the passage of drugs through the skin is the stratum corneum, the outermost layer of the skin.^{1,2,4,5} In order to enhance the permeability of drugs through the skin, many techniques have been employed to overcome stratum corneum impermeability. A popular applied technique is the use of penetration enhancers which reversibly decrease the barrier resistance of the skin.³⁻⁵ These pharmacologically inactive chemical compounds tend to interact with the stratum corneum constituents to ease the absorption of drugs through the skin by temporarily increasing in skin permeability.⁵ Trolamine salicylate is a topically applied salicylic acid derivative which is used for temporary relief of pain or inflammation in muscles, joints and other tissues below the skin.⁶⁻¹⁰ As trolamine salicylate is an odor free compound and has no skin irritant properties⁹, it can be

a viable alternative to oral salicylate but is less permeable through skin compared with other salicylic acid derivatives like methyl salicylate.¹¹ Consequently, to achieve therapeutic concentrations of salicylic acid after transdermal administration of trolamine salicylate, the application of the penetration enhancers is needed.¹² A number of penetration enhancers have been used to evaluate their influence on the in vitro permeation of trolamine salicylate through the abdominal rat skin. The results showed that the best enhancement of trolamine salicylate flux, was obtained from 12 hours skin pretreatment with Transcutol[®] (diethylene glycol monoethyl ether) and eucalyptus oil that were able to provide a near 12 and 10 fold increase in flux, respectively, in comparison with controls.¹² However, effect of skin pre-treatment with these enhancers on in vivo absorption of trolamine salicylate has not been investigated. Therefore, the aim of this study was to assess the influence of pre-treatment with Transcutol[®] and eucalyptus oil on percutaneous absorption of trolamine salicylate in rat. To do this, both non-compartmental and non-linear mixed effect modeling approaches were used to evaluate trolamine salicylate pharmacokinetics after its topical administration in rats with Transcutol[®] and eucalyptus oil pre-treated skin.

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Materials and Methods

Chemicals

Trolamine salicylate, potassium dihydrogen phosphate, perchloric acid and acetonitrile (HPLC-grade) were purchased from Merck, Germany. Eucalyptus oil, containing 70% 1,8 – cineole was from Barij Essence Company, Kashan, Iran. Transcutol®P (diethyleneglycol monoethyl ether) was kindly donated by Gattefosse, France.

Animal Experiments and Drug Administration

The study was approved by the ethics committee of the Vice Chancellor for Research and Technology of Ahvaz Jundishapur University of Medical Sciences. In vivo experiment was done on male wistar rats weighing 235 ± 20 g which supplied by Animals Care and Breeding Center of Ahvaz Jundishapur University of Medical Sciences. Twelve hours before application of penetration enhancers (one day prior to dosing), the hair of the abdominal region was removed with electric hair clippers. The rats were divided into three groups. In pre-treated groups Transcutol® (N=10) or eucalyptus oil (N=5) in the form of closed dressing were applied to the hairless surface of abdominal skin for 12 hours. Subsequently, the closed dressing was removed and the pre-treated site was carefully wiped clean 50 times with cotton to remove the excess solution and then 1 gram of 10% trolamine salicylate (PERRIGO®, USA) was applied to the pre-treated skin. In control group (N=7), trolamine salicylate was applied to the not-treated skin. Application area of depilated abdominal skin was 12 cm^2 ($3 \times 4 \text{ cm}$). Rats were under sleep condition with 140 mg/kg intra-peritoneal phenobarbital during the period of pretreatment, application of drug and blood sampling. Blood samples were collected from a heparinized catheter inserted into the tail vein at 0.5, 1, 2, 4, 6, 8 and 10 hours (when it was possible) after administration of drug. Plasma samples were immediately separated by centrifugation at 13,000 rpm for 5 minutes and were stored at -70°C until the time of analysis.

Analytical Procedure

Salicylic acid concentration in rat plasma following topical administration of trolamine salicylate was determined using high-performance liquid chromatography (HPLC) with fluorescence detection as previously reported with some modifications.¹³ The HPLC apparatus consisted of an Agilent 1260 Infinity quaternary pump and Agilent 1260 Infinity fluorescence detector (Agilent, USA). An Alltech Altima® C18 column ($150 \text{ mm} \times 2.1 \text{ mm}$, $5 \mu\text{m}$ particle size) (Grace Davison Discovery Sciences, USA) was used for the separation. The mobile phase consisted of acetonitrile: phosphate buffer (17: 83) (pH 3) delivered at a flow rate of 0.5 ml/min. Fluorescence detection was performed at 297 nm (excitation) and 407 nm (emission). Plasma samples ($50 \mu\text{L}$) were transferred into a 1.5-ml micro-centrifuge tubes and mixed with

$100 \mu\text{L}$ of perchloric acid 35%. After vortex mixing for 1 min, $300 \mu\text{L}$ of acetonitrile was added to this solution. The contents were vortex mixed thoroughly for 2 minutes and centrifuged at 1500 g for 5 minutes. Twenty μL of the clear supernatant was injected onto the column.

Pharmacokinetic Analysis

Non-Compartmental Analysis

Area under the salicylic acid plasma concentration time curve between 0 and 8 hours post administration of trolamine salicylate ($\text{AUC}_{0-8 \text{ hr}}$) was calculated using trapezoidal rule to compare the extent of absorption in rats with Transcutol® or eucalyptus oil pre-treated skin with that of the control group. Kruskal-Wallis test followed by pairwise multiple comparisons (SPSS Statistics 20, IBM, USA) was used to check any significant difference of AUC values between treatment groups.

Non-linear mixed effect modeling: Salicylic acid plasma concentration –time data were modeled by a one-compartment model with zero-order absorption input. The inter-animal error terms for all structural model parameters including T_k0 (duration of zero order absorption), k (first order elimination constant) and V/F (extravascular apparent volume of distribution) were assumed to be independently and log-normally distributed with mean zero and variance ω^2 . A constant error model was used for the residual random variability. Covariate analysis was also done to assess the effect of skin pre-treatment on trolamine salicylate pharmacokinetics. Influence of pre-treatment on the pharmacokinetic parameters were modeled using the following general equation:¹⁴

$$\ln P_{i, \text{pre-treated}} = \ln P_{i, \text{control}} + \beta_{i, \text{pre-treatment}}$$

in which $P_{i, \text{pre-treated}}$ and $P_{i, \text{control}}$ are the population values of pharmacokinetic parameter i in each of pre-treated groups and control group, respectively. β_i is the pre-treatment effect for parameter i . Non-linear mixed effect analysis was carried out using Monolix 4.2.0 (Lixoft, France). Model selection was based on the significant reduction in minimum objective function (MOF) that is equal to $-2 \times \log$ likelihood value; parameter precision (expressed as relative standard errors of the estimated parameters) and visual inspection of goodness-of-fit plots including the prediction distribution graph. Discrimination between two nested models (e.g. with and without covariate effect) was carried out using log-likelihood ratio test assuming a chi-squared distribution for the difference between minimum objective function values. A significance level of 0.005 corresponding to a decrease of 7.879 (1 degree of freedom) in minimum objective function was considered.

Results and Discussion

Salicylic Acid Analysis Method

The linear dynamic quantitation range of the employed HPLC method was between 25 (limit of quantitation)

and 5000 ng/mL in rat plasma with a correlation coefficient of 0.999. The intra- and inter-day accuracy for salicylic acid over the above concentration range fell in the ranges of 99-100 % and 110-113%, respectively with the analytical recovery of greater than 85%. The intra- and inter-day precision were 5-6% and 3-13%, respectively.

Non-Compartmental Analysis

Plasma concentration time profiles of salicylic acid following topical administration of trolamine salicylate in different groups of rats with Transcutol®- or eucalyptus oil pre-treated skin in comparison to control group (without any skin pre-treatment) is presented in Figure 1. Median values of $AUC_{0-8 \text{ hr}}$ (% of interquartile range/median) were 2522(139%), 58976(141%), 3023(327%) ng/mL/hr for Transcutol®, eucalyptus oil and control groups, respectively. As could be seen from Figure 2, significant differences were observed between median value of $AUC_{0-8 \text{ hr}}$ in rats which their skins were pre-treated with eucalyptus oil with those of Transcutol® ($p = 0.030$) and control ($p = 0.004$) groups. However, no statistically significant difference was detected between the median of $AUC_{0-8 \text{ hr}}$ in control and Transcutol® pre-treated rats. Furthermore, the inter-animal variability in $AUC_{0-8 \text{ hr}}$ was lower in rats with either Transcutol® or eucalyptus oil pre-treated skin as compared to control group.

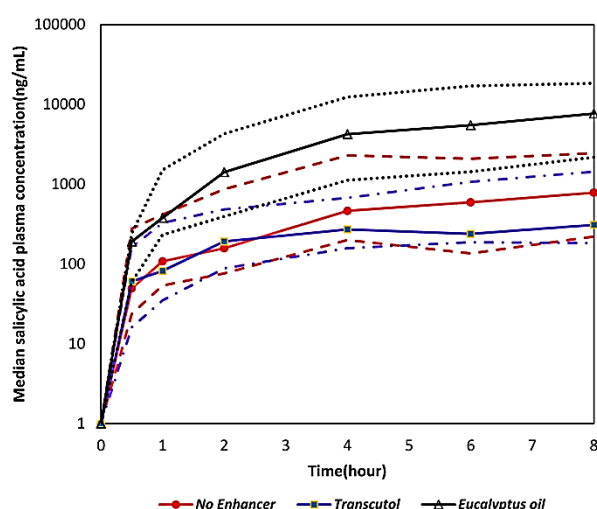


Figure 1. Median plasma concentration-time profile of salicylic acid following topical administration of trolamine salicylate in different groups of rats with Transcutol- or eucalyptus oil pre-treated skin in comparison to control group (dashed lines with the same color represent 95 % confidence around the median values).

Non-Linear Mixed Effect Modeling

Any attempt to estimate salicylic acid elimination rate constant (k) with enough precision was unsuccessful that could be related to insufficient plasma concentration-time data of the elimination phase in the majority of the rats under investigation. Therefore, the population value of k was fixed to 0.105 hr^{-1} which has been reported by Varma *et al* in a group of male rats

with the same range of age and weight.¹⁵ Parameters of the base population model (model without including any covariates) are presented in Table 1. Bayesian individual rats' estimates of pharmacokinetic parameters were used for covariate screening. As could be seen from Figure 3, statistically significant differences were observed between the median of individual estimates of the pharmacokinetic parameters in eucalyptus oil pre-treated rats with both Transcutol pre-treated and control groups. So, the influence of pre-treatment on the pharmacokinetic parameters of salicylic acid was further assessed by including it as a categorical covariate in the population model. Results of covariate analysis are shown in Table 2. Although, inclusion of both pre-treatments (with Transcutol and eucalyptus oil) as an influential covariate on all pharmacokinetic parameters led to statistically significant reductions in MOF values, the estimated β coefficients were not enough precise (p -values greater than 0.05). In case of model with pre-treatment effect on Tk_0 alone (second model in Table 2), the estimated values of Tk_0 did not make sense (4 hour for control group).

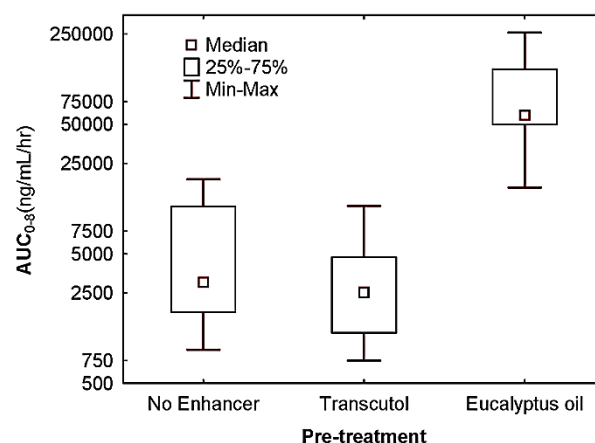


Figure 2. Comparison of area under the salicylic acid plasma concentration-time curve up to 8 hours ($AUC_{0-8 \text{ hr}}$) post administration of 100 mg trolamine salicylate (1g of 10% cream) in rats with untreated, Transcutol or eucalyptus oil pre-treated skin.

Table 1. Pharmacokinetic parameters of salicylic acid base population model (model without pre-treatment effect as a categorical covariate) following topical administration of trolamine salicylate in rats with Transcutol- or eucalyptus oil pre-treated skin and control group.

Parameter	Value	^a SE	Relative SE (%)
$Tk_0(\text{hr})$	13.8	3.1	23
$V/F(\text{mL})$	13.2	4.5	35
^b $k(\text{hr}^{-1})$	0.105	-	-
^c ω_{Tk_0}	0.53	0.17	32
^c ω_V	1.13	0.26	23
^c ω_k	2.55	0.56	22
Residual error(ng/mL)	413	24	6

^a Standard error of estimate

^b Not estimated

^c Inter-individual variability of pharmacokinetic parameters in log-normal domain

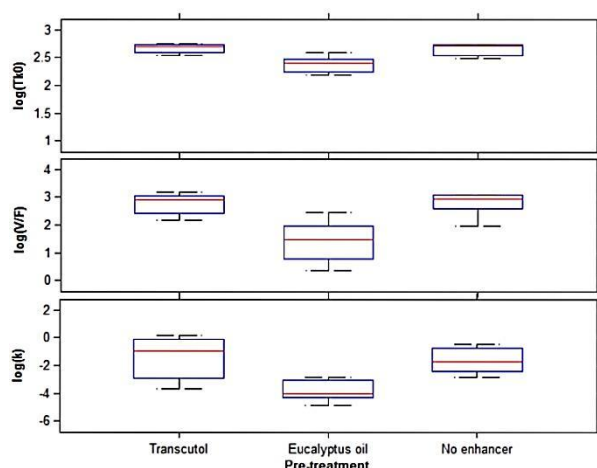


Figure 3. Comparison of base model (without pre-treatment effect as a covariate) individual estimates of salicylic acid pharmacokinetic parameters after topical administration of trolamine salicylate between different groups of rats with and without skin pre-treatment. Box and horizontal line inside it represents 25 - 75 percentile range and median, respectively. Non-outlier minimum and maximum observed values are shown by whiskers.

As could be seen from Table 2, effect of skin pre-treatment with Transcutol on pharmacokinetic parameters of salicylic acid was not statistically significant. Therefore, in the next step of covariate analysis, Transcutol® and control group rats were pooled into one group as not-treated with eucalyptus oil. The final selected covariate model (shown in italic letters) was the one that assumes a significant effect of skin pre-treatment with eucalyptus oil on extravascular volume of distribution and elimination rate constant of salicylic acid. Considering a covariance between Tk_0 and V/F resulted in better estimates of all parameters. Prediction distribution of salicylic acid plasma concentration by the final population model along with the observed data are shown in Figure 4. The majority of the observed concentration data lie within the 90 percent confidence interval of the model prediction.

Table 2. Comparison of different models with skin pre-treatment as a covariate (levels of significant difference of the β parameters are given in parenthesis).

Model	-2log-likelihood (^a MOF)	^b Δ MOF	^c β_1	^d β_2	^e β_3	^f β_4	^g β_5	^h β_6
Base model(no-covariate effect)	2870.56	-	-	-	-	-	-	-
Effect of pre-treatment on Tk_0	2833.61	-36.95	2.19 (0.0019)	0.74 (<0.0001)	-	-	-	-
Effect of pre-treatment on V/F	2844.93	-25.63	-	-	0.12 (0.75)	-2.02 (<0.0001)	-	-
Effect of pre-treatment on k	2853.97	-16.59	-	-	-	-	-0.70 (0.53)	-1.73 (0.0014)
Effect of pre-treatment on Tk_0 and V/F	2840.70	-29.86	0.70 (ⁱ -)	0.07 (0.87)	-0.22 (0.69)	-1.98 (<0.0001)	-	-
Effect of pre-treatment on Tk_0 and k	2846.82	-23.74	1.39 (0.0520)	1.13 (0.11)	-	-	-2.86 (0.77)	-1.53 (0.0001)
Effect of pre-treatment on V/F and k	2834.02	-36.54	-	-	0.36 (0.21)	-1.83 (<0.0001)	-1.99 (0.65)	-1.16 (<0.0001)
Effect of pre-treatment on Tk_0 , V/F and k	2831.93	-38.63	1.32 (1)	-0.24 (0.71)	-0.52 (0.63)	-1.79 (<0.0001)	-0.53 (0.63)	-1.26 (<0.0001)
^j Effect of eucalyptus oil on Tk_0	2860.15	-10.41	-	-1.48 (0.021)	-	-	-	-
^j Effect of eucalyptus oil on V/F	2844.56	-26.00	-	-	-	-2.01 (<0.0001)	-	-
^j Effect of eucalyptus oil on k	2853.51	-17.05	-	-	-	-	-	-1.89 (0.0005)
^j Effect of eucalyptus oil on V/F and k	2834.62	-35.94	-	-	-	-1.98 (<0.0001)	-	-1.96 (0.012)
^j Effect of eucalyptus oil on Tk_0 and V/F	2841.62	-28.94	-	-0.11 (0.84)	-	-2.03 (<0.0001)	-	-
^j Effect of eucalyptus oil on Tk_0 and k	2849.24	-21.32	-	-1.73 (0.007)	-	-	-	-1.48 (<0.0001)
^j Effect of eucalyptus oil on Tk_0 , V/F and k	2832.75	-37.81	-	-0.46 (0.42)	-	-1.85 (<0.0001)	-	-1.31 (0.0001)
^{j, k} Effect of eucalyptus oil on V/F and k assuming a covariance between Tk_0 and V/F	2833.48	-37.08	-	-	-	-2.20 (<0.0001)	-	-1.63 (0.0015)

^a Minimum objective function; ^b in comparison to the base model; ^c coefficient of the effect of Transcutol pre-treatment on Tk_0 ; ^d coefficient of the effect of eucalyptus oil pre-treatment on Tk_0 ; ^e coefficient of the effect of Transcutol pre-treatment on V/F ; ^f coefficient of the effect of eucalyptus oil pre-treatment on V/F ; ^g coefficient of the effect of Transcutol pre-treatment on k ; ^h coefficient of the effect of eucalyptus oil pre-treatment on k ; ⁱ could not be calculated; assuming no difference between pharmacokinetic parameters of rats in Transcutol and control groups and putting them into one group as not-treated with eucalyptus oil; ^k final selected model

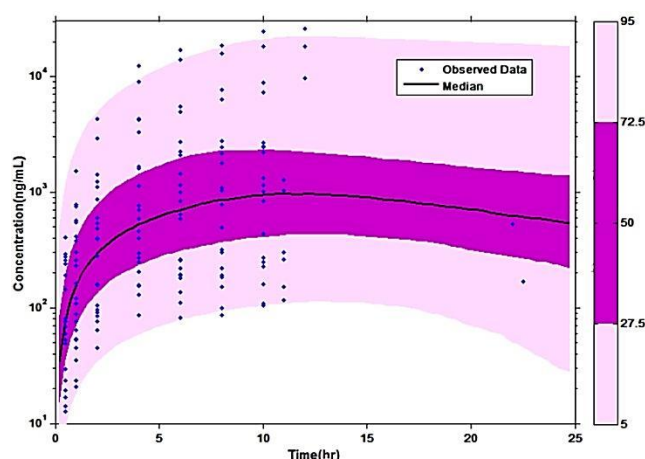


Figure 4. Prediction distribution of salicylic acid plasma concentration following administration of trolamine salicylate to rats by the final population model including skin pre-treatment with eucalyptus oil as an influential covariate (color bands represent different prediction percentiles)

Parameters of the final selected model are presented in Table 3. Results indicated that inclusion of pre-treatment with eucalyptus oil could lead to substantial reduction in inter-animal variability of pharmacokinetic parameters.

Table 3. Pharmacokinetic parameters of salicylic acid final population model (model including pre-treatment with eucalyptus oil as a categorical covariate) following topical administration of trolamine salicylate in rats with Transcutol® or eucalyptus oil pre-treated skin and control group.

Parameter	Value	^a SE	Relative SE (%)
<i>Tk0</i> (hr)	17.3	3.0	17
<i>V/F</i> (mL)	24.0	4.1	17
^b $\theta_{\text{eucalyptus oil effect on } V/F}$	-2.2	0.25	11
^c <i>k</i> (hr ⁻¹)	0.105	-	-
^d $\theta_{\text{eucalyptus oil effect on } k}$	-1.63	0.51	31
^e ω_{Tk0}	0.64	0.13	21
^e $\omega_{V/F}$	0.41	0.11	26
^e ω_k	0.70	0.34	49
correlation between <i>Tk0</i> and <i>V/F</i>	0.81	0.17	21
<i>V/F</i> eucalyptus oil group(mL)	2.7	0.5	18
<i>V/F</i> other groups(mL)	24.0	4.1	17
<i>k</i> eucalyptus oil group(hr ⁻¹)	0.021	0.01	51
<i>k</i> other groups(hr ⁻¹)	0.105	-	-
Residual error(ng/mL)	416	24	6

^a Standard error of estimate
^b Significant at *p* < 0.0001
^c Not estimated
^d Significant at *p* = 0.0015
^e Inter-animal variability of pharmacokinetic parameters in log-normal domain

Lower extravascular apparent volume of distribution in rats with eucalyptus oil pre-treated skin as compared to other groups, might be due to the enhanced bioavailability (increase in *F*) of trolamine salicylate.

On the other hand, a considerable decrease in elimination rate constant of the eucalyptus oil group rats, could be attributed to the saturation of salicylic acid elimination pathways due to the high plasma concentration of salicylic acid achieved following application of eucalyptus oil as transdermal penetration enhancer.^{16,17}

Tk0 is not affected by pre-treatment, therefore the zero order absorption rate is not different among three groups of rats.

Permeation Enhancing Effect of Transcutol® and Eucalyptus Oil

The results of the present study show that eucalyptus oil created an appreciable increase in transdermal absorption of trolamine salicylate through rat skin compared with the control while Transcutol® did not have any significant enhancing effect on trolamine salicylate permeation.

The inability of Transcutol® to promote transdermal permeation of trolamine salicylate in the present study is inconsistent with the finding of *in vitro* study of Sharif Makhmalzade and Hasani.¹² In their experiments Transcutol® was found to cause the best enhancement of trolamine salicylate flux (12 fold) followed by eucalyptus oil (10 fold) in comparison to control. Such a lack of correlation between *in vitro* and *in vivo* permeability of triethanolamine salicylate was also reported by Cross *et al* during their study on topical penetration of salicylate esters and salts using human isolated skin and clinical microdialysis technique. They suggested that there is a possibility that *in vivo* salicylate in the epidermis could not be released as quickly as *in vitro* because of differences in sink condition or some sorts of strong binding to tissue constituents.¹⁸

According to the contradictory results that have been published in the literature, Transcutol® can increase or decrease transdermal delivery of topically applied compounds. It has been reported that prostaglandin¹⁹ and theophylline²⁰ *in vitro* skin permeability was increased by the presence of Transcutol® due to its solubilizing effect that increases the solubility of drug in the skin, thus provides a raising in drug partitioning.²¹ However, other researchers found that Transcutol® was not able to show any significant enhancing effect on the percutaneous absorption of morphine,²² salicylic acid²³ and melatonin.²⁴

It has been suggested that Transcutol® can lead to formation of cutaneous depot of drugs due to its ability to cause intercellular lipids swelling, thereby associated with drug entrapment in the skin.²⁵⁻²⁷ In addition, high molecular weight of some drugs have been suggested to be one of the restrictions for Transcutol® effectiveness.²⁸

1, 8 Cineol (eucalyptol) is the principal chemical component of eucalyptus oil. Cineol is a cyclic ether and tend to be more effective on hydrophilic drug (like salicylates) permeation. This penetration enhancer

affects the lipid bilayer structure by forming liquid pools in those region. Thus cineol increases skin permeability by disrupting the lipid structure of the stratum corneum.^{5,29,30}

Conclusion

Unlike eucalyptus oil, skin pre-treatment with Transcutol® could not lead to enhancement of trolamine salicylate transdermal absorption in rat. Eucalyptus oil could result in more than 20 fold increase in systemic absorption of trolamine salicylate through rat skin as compared to control group.

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Conflict of Interest

There is no conflict of interest to be reported.

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