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Research Article

The Effect of Chemical and Physical Enhancers on Trolamine Salicylate Permeation through Rat Skin

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Abstract

Purpose: *To achieve percutaneous delivery of trolamine salicylate to muscle and joints for the treatment of inflammatory muscle, tendon and joint diseases.*
Methods: *Trolamine salicylate permeability parameters through rat skin were evaluated with and without chemical enhancers - Transcutol, eucalyptus oil, oleic acid and sodium lauryl sulfate – using the permeability cell technique.*
Results: *The main barrier for trolamine salicylate permeability was the epidermis layer of the skin. Also, partitioning from the aqueous donor phase into the skin was the rate-limiting step for drug flux. Transcutol and eucalyptus oil were the most effective enhancers as they increased flux 11-fold. Sodium lauryl sulfate disrupted the lipid structure of the skin and thus increased diffusion coefficient 3-fold. Supersaturation technique did not increase flux. Propylene glycol in cosolvent system increased drug solubility in donor phase and partitioning.*
Conclusion: *Trolamine salicylate exhibited less flux and diffusion coefficient through rat skin than salicylic acid due to its hydrophilic property. Partitioning from vehicle into skin was the rate-limiting step for trolamine salicylate permeability through rat skin.*

Keywords: *Trolamine salicylate, Percutaneous absorption, Chemical enhancers, Supersaturation technique, Differential scanning calorimetry*

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INTRODUCTION

Permeation of drugs through the skin is the basis of transdermal delivery [1]. Transdermal drug delivery is associated with some advantages such as controlled drug delivery, continuous drug delivery (which is important for drugs with short biological half-life and low therapeutic indices), first-pass intestinal and hepatic bypass, avoidance of the gastrointestinal irritation (which is common with oral medications such as salicylates), and facilitation of drug localization at target site [1] Percutaneous delivery of salicylates to muscle and joints is the goal in the treatment of inflammatory muscle, tendon and joint diseases tendon and joint diseases [2].

The two main steps in skin penetration are partitioning and diffusion through the stratum corneum, partitioning and diffusion to the viable epidermis, passage into the dermis and finally, systemic absorption or penetration into deeper tissues. The greatest barrier to drug penetration is the stratum corneum, the outermost layer of the skin [3]. The stratum corneum poses a formidable challenge to drug delivery systems. Several approaches have been used to improve entry of drugs into lower skin layer and deeper tissues. Chemical and physical permeation enhancers have been designed to facilitate delivery of high drug concentrations across the skin into systemic circulation or deeper tissues [4]. The classes of enhancers used and the mode of action of these agents vary [5]. Increased drug diffusivity in the skin, stratum corneum lipid fluidization, increase in thermodynamic activity of drug in the skin and vehicle, as well as effect on drug partition coefficient, are the most common modes of action of chemical enhancers. Supersaturation is a physical enhancement technique that involves increasing the thermodynamic activity beyond the saturation level. This technique is inexpensive and does not alter the integrity of skin [6].

Trolamine salicylate is a compound formed from trolamine and salicylic acid and is applied to relieve pain in muscles, joint, tendons and non-articular musculoskeletal conditions [7]. Compared with methyl salicylate, trolamine salicylate is an odourless compound but is less permeable through skin than the former [2,8]. For effective treatment, trolamine salicylate has to penetrate the skin into muscles and joints. In the past, it was believed that drugs that pass through the epidermis and dermis is effectively removed by cutaneous microcirculation but later studies have shown that local subcutaneous drug delivery is not only feasible but can be effective [9].

The aim of this study, therefore, is to develop a transdermal trolamine salicylate delivery system and evaluate the effect of chemical and physical enhancers on its permeation characteristics through rat skin.

EXPERIMENTAL

Materials

Trolamine salicylate was purchased from Chemos GmbH, Germany. Eucalyptus oil, containing 70 % 1,8-cineole, was obtained from Barij Essence Iranian Company, Kashan, Iran while oleic acid, sodium lauryl sulfate and propylene glycol were supplied by Merck. Ethoxydiglycol (Transcutol CG) was kindly donated by Gattefosse Faratin Company, Tehran, Iran, while potassium phosphate monobasic was purchased from Sigma. Water was deionized and filtered in-house. All other chemicals and reagents used were of analytical grade.

Animal experiments

Male wistar rats weighing 260-340 g were used for the *in vitro* permeation study. After sacrificing under ether anaesthesia, the abdominal skin hair was carefully removed with an electric clipper and razor without damaging the skin. The skin was excised and any extraneous subcutaneous fat was removed from the dermal surface. Whole skin thickness was measured using a digital

micrometer (AACO, France). The epidermis was obtained by a heat separation method. In this technique, the whole skin was soaked in water at 60 ^{o}C for 1 min, followed by careful removal of the epidermis [10]. The animals were treated according to the principles for the care and use of laboratory animals, and approval for the studies was given by the Ethical Committee of Ahvaz Jundishapour University of Medical Sciences (ref no. 4371). The guidelines followed were those laid down by the National Academy of Sciences and published by the National Institutes of Health, USA.

Solubility determination

The solubility of trolamine salicylate and salicylic acid in water, buffer and propylene glycol solutions was studied by equilibrating the suspension of excess amount of drug in 5 ml of medium and shaken gently for 24 h at 32 ^{0}C. It was then centrifuged for 10 min at 3000 rpm, filtered, diluted and analyzed by UV spectrophotometry (Cecil, England).

***In vitro* permeation study**

Diffusion cells fabricated in-house and with an effective area of approximately 2.49 cm^2, were used for the permeation studies. Whole skin and epidermis samples were placed between donor and receptor chambers of the cells with the epidermal side facing the donor compartment. The skin samples were hydrated prior to use. The donor phase was filled with 3 ml saturated aqueous solution of drug while the receptor compartment was filled with phosphate buffer (pH 7). Trolamine salicylate is soluble in this medium which also provides perfect sink conditions. Temperature was maintained at 37 ± 0.5 ^{0}C and the receptor chamber was stirred at 300 rpm. At predetermined time intervals, 2 ml of the receptor medium was withdrawn and immediately replaced with an equal volume of fresh buffer. The permeated amount of trolamine salicylate was determined by UV spectrometry at 276 nm. Aqueous saturated solution of salicylic acid was used as control.

Effect of chemical enhancers on drug permeation across rat skin

Fully hydrated samples were used as controls. To minimize experimental errors arising from biological variability, each piece of skin was used as its own control. For pretreatment of skin samples, fully hydrated samples were pre-treated by placing 1 ml of chemical enhancer on the surface of the skin in the donor phase. The donor and receptor chambers were then washed with water and filled with aqueous saturated solution of trolamine salicylate and phosphate buffer (pH 7), respectively. The effect of chemical enhancers was evaluated for trolamine salicylate permeation only through full skin samples. Transcutol, olive oil (containing 70 % 1,8-cineole) and eucalyptus oil were used as received without dilution while sodium lauryl sulfate was used as 1 % aqueous solution.

Evaluation of the effect of supersaturated solution on skin permeation

The solubility of trolamine salicylate in varying ratios of propylene glycol/water mixture (30/70, 50/50 and 70/30) was determined by shaking the drug in the mixture. The mixture was then filtered through a nylon filter and analyzed by UV spectroscopy at 276 nm after appropriate dilution. Trolamine salicylate solution (50 and 70 %)) in propylene glycol (PG) was mixed with water. A saturated solubility plot of trolamine salicylate in PG/water cosolvent system was drawn at 32 ^{0}C. The effect of these two supersaturated solutions on drug permeation through the whole rat skin was compared with that of the saturated solution of trolamine salicylate in water which served as control.

Differential scanning calorimeter (DSC)

DSC studies were carried out with a Mettler DSC facility (model CH 8603). The fully hydrated skin samples were first immersed in a chemical enhancer for 16 h and the excess

of the enhancer was blotted out before they were hermetically sealed to avoid evaporation of water. The scan rate was 10 ℃/min in the temperature range 25 - 150 ℃. Enthalpies (∆H) were calculated from the endothermic transitions of the thermograms as in Eq 1 [11]:

∆H = peak area/sample weight …… (1)

Data analysis

The cumulative amount of trolamine salicylate penetrating a unit area of the diffusion surface into the receptor was calculated and plotted as a function of time. Flux (J) was calculated from the slope of the linear portion of the penetration curves and expressed as the mass of drug passing across 1 cm^2 of skin time. Steady state drug diffusion from a saturated solution through a skin membrane is represented as in Eq 2.

J = PS = (KD/h)S …… (2)

where J is the flux of the drug and P is the permeability coefficient comprising of the membrane/vehicle partition coefficient (K), diffusivity coefficient (D) and skin thickness (h). P was calculated by dividing J by the drug's saturated solubility (S) in the donor phase [12]. Enhancement ratios were calculated from the ratio of permeation parameters after enhancer treatment to that for controls.

The statistical significance of the difference between various treatments was determined using one-way ANOVA. Differences were considered to be statistically significant at $p < 0.05$. Correlation analyses were performed by least square linear regression method and correlation coefficients examined for significance by Student's t - test. All statistical analyses were conducted using SSPS software (SPSS 13.0 for Windows, SPSS Inc, Chicago, IL, USA)

RESULTS

Trolamine salicylate permeability

The permeability properties of trolamine salicylate through whole skin and epidermis, and salicylic acid through whole skin, expressed as flux, apparent diffusion coefficient (D) and lag time (t_{lag}), are shown in Table 1.

Table 1: *In vitro* permeability parameters for trolamine salicylate and salicylic acid (mean ± SD, n=5)

Parameter	Trolamine salicylate		Salicylic acid
	Whole skin	***Epidermis***	***Whole skin***
Flux ($mg.cm^{-2}.h^{-1}$)	3.01 ± 0.28	15.88 ± 1.75	3.95 ± 0.41
D ($cm^2.h^{-1}$)	$4.1\times 10^{-3} \pm 3\times 10^{-4}$	$4.5\times 10^{-3} \pm 4\times 10^{-4}$	$5.2\times 10^{-2} \pm 4\times 10^{-3}$
T_{lag} (h)	4.15 ± 0.53	2.27± 0.2	1.95± 0.18

The results showed that the flux of trolamine salicylate through epidermis was significantly higher ($p < 0.05$) than through whole skin but T_{lag} was significantly lower ($p < 0.05$). Both trolamine salicylate diffusion coefficient and solubility in whole skin were the same as in the epidermis.

Effect of chemical enhancers on trolamine salicylate permeability

The effect of chemical enhancers on trolamine salicylate permeability is presented in Table 2 as ER_{flux} (ratio of drug flux after and before skin pretreatment with enhancer) and ER_D (drug diffusion coefficient after and before skin pretreatment with enhancer). Hydrated skin with no enhancer pretreatment and aqueous saturated solution of trolamine salicylate as donor phase served as control. The results indicate that eucalyptus oil, Transcutol and sodium lauryl sulfate increased trolamine salicylate flux and diffusion coefficient significantly ($p < 0.05$) but oleic acid only increased diffusion coefficient significantly ($p < 0.05$). Transcutol provided

the best enhancement of trolamine salicylate flux, increasing it approximately 12-fold relative to control, followed by eucalyptus oil (10-fold) and sodium lauryl sulfate (1.25-fold). All the chemical enhancers exerted significant effects on diffusion coefficient ($p < 0.05$), with sodium lauryl sulfate showing the greatest enhancement effect on diffusion coefficient (3 – fold).

Table 2: Effect of chemical enhancers on derived permeation parameters for trolamine salicylate through whole skin (mean ± standard deviation, n = 5)

Chemical enhancer	ER_{flux}	ER_D
Oleic acid	0.95±0.07	1.56±0.17
Sodium lauryl sulfate	1.24±0.11	3.40±0.29
Eucalyptus oil	10.03±0.37	1.98±0.12
Transcutol CG	11.73±0.82	1.89±0.14

ER_{flux} *= ratio of flux after and before treatment with enhancer;* ER_D *= ratio of diffusion coefficient after and before treatment with enhancer*

Effect of supersaturation on trolamine salicylate permeability

Trolamine salicylate solubility in water (71.8 mg/ml) increased with increase in propylene glycol (PG) content, increasing 2-fold to 150.25 mg/ml.

The permeability parameters of the two supersaturated solutions selected (with degree of saturaturation of 1.12 and 1.43, respectively) are indicated in Table 3. The higher the degree of saturation (DS) the greater was the flux; however, flux in saturated solution was higher than in supersaturated solution. On the other hand, there was no difference between the diffusion coefficients in supersaturated and saturated solutions. Crystallization time for the supersaturated solution was > 7 days. The supersaturated solutions were translucent and without crystals during the permeability experiments.

DSC

The thermogram of hydrated whole rat skin is shown in Fig 1. It shows an endothermic transition at around 70.3 ± 1.4 ^{0}C with an enthalpy of 12.7 ± 0.9 (mean ± SD, n = 3) (Table 4). This transition disappeared following treatment of the skin with the lipid-extracting solvent blend, chloroform/methanol (3:1).

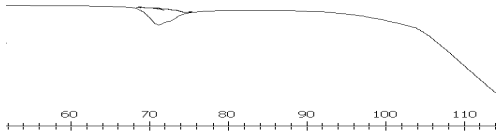

Figure 1: Thermogram of hydrated whole rat skin

Table 3: *In vitro* permeability parameters of trolamine salicylate in supersaturated and aqueous saturated solutions (mean ± SD, n=5)

Parameter	Supersaturated solution		Aqueous saturated solution
	DS=1.12	***DS=1.43***	
Flux ($mg.cm^{-2}.h^{-1}$)	1.56 ± 0.12	1.87 ±0.16	2.37 ±0.25
D $x10^{-3}$ ($cm^2.h^{-1}$)	8.6 ±0.6	8.9± 0.7	6.7±0.7
T_{lag} (h)	2.39 ± 0.26	2.26± 0.2	2.69± 0.31

Table 4: Effect of chemical enhancer on the thermal properties of hydrated rat skin (mean ± SD, n = 3).

Pretreatment	Transition temp T (℃)	ΔT (℃)	Transition enthalpy Δ_H	$\Delta_{(\Delta H)}$
FHC	70.29 ±1.44	-	12.68±0.88	-
EO	90.2 ± 1.2	+20.09	1.8 ± 0.05	-10.88
SLS	-	-	-	-
TC	93.74 ± 1.5	23.45	1.85 ± 0.07	-10.83
OA	78.31 ± 1.1	+8.02	16.1± 0.9	+3.42

FHC = Full-hydrated control; EO = Eucalyptus oil; SLS = Sodium lauryl sulfate; TC = Transcutol; OA = Oleic acid

DISCUSSION

It seems that the main barrier against trolamine salicylate penetration through rat skin is the epidermis because the difference between the diffusion coefficient of drug through whole skin and epidermis was not significant. This finding is in tandem with the

physicochemical properties of trolamine salicylate which is a hydrophilic compound. Cross *et al* [2] reported that the permeability of the methyl ester and trolamine salt of salicylate through human epidermis was 3-fold faster through whole skin. They suggested that 99.9 % of the salicylate present in whole skin samples treated with trolamine salicylate formulation could be accounted for in the epidermis and that the epidermis was the major site for deposition of salicylate from the salt. On the other hand, trolamine salicylate was less permeable than salicylic acid and thus indicated that the trolamine salt decreased salicylate permeability. Permeation through membrane consists of two processes, partitioning and diffusion. There were no significant differences in trolamine salicylate partitioning and diffusion and so it seems that the difference in the drug flux is according to membrane thickness. Trolamine salicylate is a hydrophilic compound and so its permeation through stratum corneum is the rate-limiting step. In the receptor and donor phases trolamine salicylate was completely. The permeability of salicylic acid through skin is related to pH. Permeation of salicylic acid (an anion) occurred in weakly acidic conditions (pH 4.5 - 6.5). In this study, the pH of the donor phase did not limit the permeability of salicylic acid through rat skin. On the other hand, the permeability of trolamine salicylate was comparable to that of salicylic acid. Trolamine salicylate reduced skin permeability of salicylic acid.
Trolamine salicylate is a hydrophilic compound and its partitioning and diffusion through rat skin was less than that of salicylic acid which is a less hydrophilic compound [2].

Autoradiography and labeled studies suggest that during transdermal absorption of trolamine salicylate, the salt may disassociate [13]. In another research, the flux of salicylate from methyl salicylate and trolamine salicylate formulation applied to whole rat skin was found to be 40 % higher than for trolamine salicylate [14].

Transcutol and eucalyptus oil were the most effective enhancers followed by sodium lauryl sulfate. Oleic acid was the least effective enhancer and improved diffusion coefficient only. The results suggest that partitioning from aqueous solution into skin was the rate-limiting step in skin penetration. ranscutol and eucalyptus oil, with higher drug solubility in skin, increased flux 11-fold approximately. Sodium lauryl sulfate had the greatest effect on diffusion coefficient which it increased 3-fold. The effect of sodium lauryl sulfate on skin permeability of hydrophilic compounds with log P < 3 (where P is permeability coefficient) has previously been reported [15]. Comparison between the effect of sodium lauryl sulfate on flux and diffusion coefficient suggests that sodium lauryl sulfate increased flux by enhancing diffusion coefficient; thus, the effect on partitioning phenomena was not important.

The effect of Transcutol (ethoxydiglycol) and eucalyptus oil on flux was significantly higher than on diffusion coefficient. This observation indicates that the main mechanism of action of Transcutol and eucalyptus enhancement activity is facilitation of drug partitioning into skin. Transcutol is a powerful solubilizing agent and is miscible with polar and non-polar solvents [16]. In the present study, it seems that ethoxydiglycol increased the solubilizing affinity of the skin for trolamine salicylate, thus increasing its partitioning. Eucalyptus oil consists of 75 % 1,8- cineole. Cineole is a cyclic terpene that acts by creating liquid pools in stratum corneum and disrupting the lipid structure of the stratum corneum, thereby increasing the diffusion coefficient of polar drugs in the membrane [17]. It is capable of forming complexes with hydrophilic compounds by hydrogen bonding. Therefore, it seems that cineole, by disrupting the lipid structure of the stratum corneum, increased the diffusion coefficient of trolamine salicylate but the effect of cineole on flux was not limited to diffusion coefficient. Apparently, complex formation and alteration in the solubility properties of stratum corneum also increased the partitioning of trolamine

salicylate from the aqueous donor phase into skin.

The effect of oleic acid on permeability coefficient and diffusion coefficient of trolamine salicylate suggests that oleic acid decreased partitioning into skin because oleic acid is a fatty acid and increased the lipophilicity of the skin but reduced the solubility of trolamine salicylate (hydrophilic compound) in stratum corneum. This effect of oleic acid on partitioning neutralized its effect on diffusion. This finding is contrary to an earlier observed effect of oleic acid on the permeability of mannitol which is also a hydrophilic compound [18].

Thermal analysis

The thermogram of rat skin indicated only one phase transition. The main structure involved in this phase transition appeared to have been driven by thermal events related to lipids in which there was no covalent bonding since lipid structures with covalent bonding are not removable by the solvent mixture used [19]. In the literature, the main transition phase reported for hydrated rat skin occurred at 81 ^{0}C as against the 70.3 ^{0}C in our study [20]. The difference between the two values may be due to the different skin hydration levels in the two studies since the thermal behavior of lipids is influenced by skin hydration levels [19].. Following pretreatment of whole skin, sodium lauryl sulfate removed the transition phase (T_{70}), while Transcutol, eucalyptus oil and oleic acid shifted the transition temperature by +23.45 , + 20.09 and + 8.02 ^{0}C, respectively and also reduced transition enthalpy significantly.

The thermogram of skin pretreated with sodium lauryl sulfate seems to confirm the effect of the enhancers on the diffusivity of the skin in that sodium lauryl sulfate eliminated the phase transition at T_{70}. However, the effect of trolamine salicylate and eucalyptus oil on skin diffusivity and the transition phase at T_{70} were less pronounced than that of sodium lauryl sulfate. Two deductions can be made from this. First, the lipid structure related to T_{70} was the main barrier against trolamine salicylate diffusion. Second, diffusion through this lipid structure was not rate-limiting for flux because the effect of sodium lauryl sulfate on transition phase at T_{70} was more pronounced than those of ethoxydiglycol (Transcutol) and eucalyptus oil. On the other hand, the effect on flux was the other way round.

Supersaturation did not increase skin flux. The presence of propylene glycol in the cosolvent system increased drug solubility in the donor phase while lowering drug partitioning into skin. However, it would seem that propylene glycol contact time with the skin was not sufficient to alter skin barrier properties and hence supersaturation did not improve skin permeability. Consequently, the supersaturated solution did not alter the barrier properties of skin. Propylene glycol increased solubility of trolamine salicylate in the donor phase but decreased its partitioning into skin. The use of a supersaturated solution as a physical enhancement technique is thus effective for lipophilic compounds. Propylene glycol increased trolamine salicylate solubility up to 2-fold but this enhancement was not enough to increase the concentration gradient across the skin.

CONCLUSION

The main barrier against trolamine salicylate permeability is partitioning into skin and, therefore, to achieve a good percutaneous formulation with satisfactory dermal penetration, incorporation of ethoxydiglycol (Transcutol) and eucalyptus oil would be of great advantage. Supersaturation of the drug in the formulation clearly did not increase its permeability through skin. Therefore, since trolamine salicylate is a non-irritating and odourless salicylate, but is less permeable than methyl salicylate and salicylic acid, it could become a suitable candidate for the treatment of inflammatory muscle, tendon and joint diseases if it can be made to

penetrate deeply to achieve effective tissue concentrations with the aid of enhancers such as ethoxydiglycol (Transcutol) and eucalyptus oil.

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