Cineole as Skin Penetration Enhancer

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Abstract Cineole (1, 8-cineole, eucalyptol) was evaluated as a penetration enhancer towards 5-fluorouracil (5-FU) using excised rat skin. It caused a near 93 fold increase in drug permeability coefficient. The enhancer gives increased partition coefficient but the diffusion coefficient values were higher. Removal of the stratum corneum has eliminated the resistance to the drug permeation. After removal of the stratum corneum the permeability coefficients of 5-FU increased by a factor of 166 compared with full thickness skin with the stratum corneum intact, showing that main resistance to the permeation of polar drug lies in the stratum corneum. The mode of action of the accelerant may be described by combined process of partition and diffusion, the diffusion process being dominant.

Key words 5-Fluorouracil; Cineole; Skin permeation; Promoting effect

1 Introduction

Transdermal administration of many drugs is often precluded because of the stratum corneum barrier. In attempts to reduce this diffusional barrier researchers have employed penetration enhancers which usually disrupt the highly ordered membrane structure[1~5]. Cineole (1, 8-Cineole, eucalyptol), the chief constituent (approx. 80%) of Eucalyptus oil, is used as a flavor in pharmaceuticals[6]. Williams and Barry[7] studied the penetration enhancing activity of cineole towards 5-fluorouracil (5-FU) using human cadaver skin. We have investigated the penetration enhancing activity of cineole towards the permeation of 5-FU in excised rat skin which will be also helpful to check the similarity between different kinds of skin.

2 Materials and methods

2.1 *Materials*

5-FU was obtained from Shanghai 12th Pharmaceutical factory and cineole was purified in our laboratory from eucalyptus oil by fractional distillation; purity 93.5; as determined by freezing point determination method, as described by BP (1993)[8].

2.2 *Skin preparation*

Male white rats weighing 190 to 240 g were used. The animals were sacrificed and hairs from the abdominal side were clipped. The clipped abdominal skin was excised from the animal and the subcutaneous fat on the dermal side was removed. The skins were floated on normal saline for 12 h before use to ensure full tissue hydration.

The samples of full thickness skin without stratum corneum were prepared by the tape stripping method as described by Williams and Barry[9].

2.3 *Permeation experiments*

A two chamber cell system with a diffusional area of 2.01 cm^2 was used. Receptor cell was filled with 16.5 ml of normal saline solution and stirred at 100 r/min. The donor cell receives 1 ml of saturated solution of 5-FU. To ensure full saturation of donor solution a crystal of 5-FU was placed in donor compartment. The receiving cell

(20) *Received 13 Nov., 1998.* Supported by National Natural Science Foundation of China.

was maintained at (37± 0.5)℃, while donor cell was exposed to the ambient temperature. At appropriate time 4 ml of solution was withdrawn from the receptor compartment and 1 ml was used for analysis by HPLC. After sampling, 4 ml of fresh normal saline was added to the receptor to keep the volume constant. At pseudo steady-state diffusion, the permeability coefficient (Kp) of the drug was evaluated[10]:

$$Kp = \frac{J}{C}$$

To determine the enhancing effect of the Cineole the skins were prepared in the same way and mounted between donor and receptor compartments. Here donor compartment receives 150 ul of cineole. After 12 h of treatment the excessive enhancer was removed by swabbing with tissue paper and replaced with the drug solution; the permeability coefficient at steady state was re-evaluated. An enhancement ratio (E_R) may be used to define the activity of enhancer[7]:

$$E_R = \frac{\text{Kp after application of penetration enhance}}{\text{Kp before application of penetration enhance}}$$

2.4 *Analysis of 5-FU*

Concentration of 5-FU in the receptor solution was determined by HPLC. Shimadzu LC-5A system equipped with UV detector was used. A Zorbax ODS column (4.6 mm× 25 cm) was employed. The eluting solvent was water and the flow rate was 0.8 ml/min. Detection was done at 266 nm. The retention time of 5-FU was 7.2 min.

2.5 *Skin retention*

At the end of experiment the exposed skin was cut down, blotted dry, weighed and cut into small pieces. The tissue was homogenized with 4 ml of ethyl acetate thrice. The homogenates were mixed, filtered, and ethyl acetate evaporated to dryness. The residue was reconstituted with 2 ml of water and analyzed for the drug content by HPLC.

2.6 *Solubility studies*

To determine the solubility of 5-FU in cineole an excess of 5-FU was added to 5 ml of cineole, heated to (37± 0.5)℃, vortex stirred and equilibrated for 24 h. A 0.5 ml of sample was taken, filtered through 0.8 μm filter, diluted to 10 ml with ethyl acetate and analyzed by HPLC.

3 Results and discussion

The mean permeability coefficients of 5-FU before and after treatment of the skin with cineole are shown in Tab 1 and indicated the obvious enhancing effect.

Tab 1. Mean Flux, Permeability coefficients (K_P), Enhancement ratios (E_R), of 5-FU in rat skin before and after treatment with cineole ($\bar{x}\pm s$, n= 5)

Enhancer	Flux μg/cm²/h	K_P cm/h(× 10^{-3})	E_R
Control	13.0± 0.28	1.053± 0.02	–
Cineole	1223.7± 12.4	97.8± 0.19	92.87
Stripped control	2188.4± 12.6	175.07± 1.0	166.25
Stripped treated	2590± 16.8	207.2± 11.3	196.77(1.183)

In Fig 1, the total amount of penetrant that appeared in the receptor fluid is plotted as a function of time. After skin treatment with a enhancer, the lag time for 5-FU falls. The steady state conditions were observed only for 4~ 6 h, when cineole was used as enhancers, and when stripped stratum corneum was used the steady state permeation was observed only for 2~ 3 h, which may contribute to accumulation of higher concentrations of 5-FU in receiver compartment and/or the wash up effect of the enhancer in diffusion cell. Cineole clearly increased drug permeation across the skin, E_R being 92.87 (P> 0.01), Tab 1.

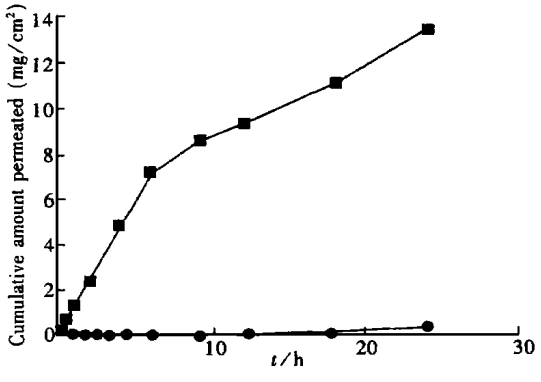

Fig 1. Effect of the cineole on the permeation of 5-FU across rat skin (—●— control; —◇— treated)

Removing the stratum corneum completely eliminated the barrier properties of the skin. Permeation profiles of 5-FU through stripped stratum corneum with and without the cineole pretreatment are shown in Fig 2. A little difference

was observed between the treated and untreated stripped membranes, as E_R values obtained were 166.25 and 196.77, respectively, when the full skin was taken as a control. When the stripped untreated membrane was taken as a control E_R for treated stripped membrane was 1.18 only.

Partitioning results are also given in Tab 2. Treatment of skin with cineole generally increased partitioning of the drug into the skin as illustrated by the partition ratio, P_R[7], where

$$P_R = \frac{P_C \text{ after enhancer treatment}}{P_C \text{ before enhancer treatment}}$$

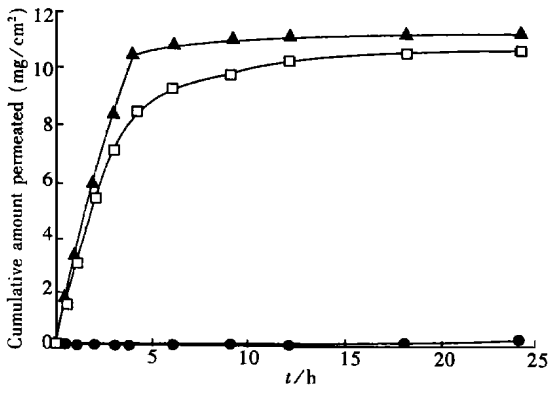

Fig 2. Typical permeation profiles of 5-FU through stripped stratum corneum rat skin (—●—) full thickness; (—□—) stripped untreated; (—▲—) stripped treated

Tab 2. Mean Partition coefficient (P_C), Partition ratios (P_R), Diffusion coefficients (D), and diffusion ratios (D_R) of 5-FU into fully hydrated skins ($\bar{x} \pm s$, $n = 5$)

Enhancer	P_C $\times 10^{-3}$	D cm/h((10^{-5})	P_R	D_R
Control	46.94± 4.3	6.7± 0.4	1	–
Cineole	115.4± 5.3	253.0± 1.5	2.45	37.761
Stripped control	7.47± 0.1	7020± 41	0.15	1047.46
Stripped treated	18.36± 3.5	3385± 38	0.39(2.45)	505.22 (0.482)

The partition ratio obtained by treating the skin with cineole was more than double (P_R= 2.45). The partition ratios obtained with stripped untreated and stripped treated skin were less than the unity when full thickness skin was taken as a control, and more than double when stripped untreated membrane was taken as a control.

Using the experimentally determined partition coefficients, a diffusion coefficient (D) of 5-FU in skin may be calculated by[7]:

$$D = \frac{K_p \cdot h}{P_C}$$

Where Kp is the mean permeability coefficient and h is the thickness of hydrated stratum corneum (taken as 3×10^{-3})[7]. From diffusion coefficients value (Tab 2), it can be seen that the use of enhancer has increased the resistance to the diffusion of the drug as mean untreated diffusion coefficient value of 5-FU is 6.76± 0.4× 10^{-5} cm^2/h. only. The increased diffusivity of 5-FU in the skin may be expressed as a diffusivity ratio, D_R[7]:

$$D_R = \frac{\text{diffusivity of 5-FU after treatment}}{\text{diffusivity of 5-FU before treatment}}$$

The solubility of 5-FU in cineole, as determined, is about 0.043 mg/ml. 5-FU is much less soluble in enhancer than in the donor phase, water. Solubility of 5-FU in water is 12.5 mg/ml[11], therefore, the enhanced permeation of 5-FU is not related with its solubility, but only with efficiency of enhancer on skin.

Some essential oils and their terpene constituents have recently been investigated as potential enhancers. Eucalyptus oil has been used to promote the percutaneous absorption of nicotine[12], and 5-FU[13, 14], and cineole of benzocaine, procaine, bupranolol, indomethacin, dibucaine[15], and 5-FU[7]. In present study Cineole was found to be very active, no any lag time was observed. The enhancer increased the penetration of 5-FU by aprox. 93 fold, and this is in agreement with Williams and Barry[7], who found that

1,8-cineole caused a 95 fold increase in 5-FU permeability through human cadaver skin.

Removal of the stratum corneum has completely eliminated the resistance to the drug permeation. The partition ratios calculated suggest that the enhancer increases partition of the drug into skin. As the drug is less soluble in enhancer than water, therefore this increased partition of drug may be retention of the drug by skin, as in this study full thickness skin was used to determine drug contents. Therefore enhanced permeation of 5-FU may be not only by increasing the partition of the drug in to stratum corneum, but also by modifying intercellular lipids, disrupting their highly ordered structure, thus increasing the diffusion of the drug through skin, as observed by comparatively increased diffusion coefficient values.

This study has shown that the cineole may offer a large and useful selection of relatively safe penetration enhancer to aid topical drug delivery. However, further studies are required to make the use of cineole as such or in combination with other enhancers in pharmaceutical preparations.

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皮肤渗透促进剂桉叶素的研究

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摘 要 研究了桉叶素促进 5-氟尿嘧啶渗透通过离体大鼠皮肤的作用,其增加药物渗透系数约达 93倍。桉叶素提高了分配系数,但对扩散系数的增加更大。去除角质层,药物的渗透障碍完全消失。与全皮相比,角质层去除后药物的渗透系数增加 166倍,表明影响极性药物渗透的主要障碍是角质层。促渗效果可能是扩散和分配综合作用的结果,其中扩散是主要作用。

关键词 5-氟尿嘧啶; 桉叶素; 皮肤渗透; 促渗作用